

17064

Cultivation of Marine Organisms as a Means of Understanding Environ- mental Influences on Populations

TRYGVE BRAARUD

Institute for Marine Biology, University of Oslo, Oslo-Blindern, Norway



Vlaams Instituut voor de Zee
Flemish Marine Institute

Reprinted from OCEANOGRAPHY
AMERICAN ASSOCIATION FOR THE ADVANCEMENT OF SCIENCE
Copyright 1961

B38

Cultivation of Marine Organisms as a Means of Understanding Environmental Influences on Populations

TRYGVE BRAARUD

Institute for Marine Biology, University of Oslo, Oslo-Blindern, Norway

AT a symposium on "Perspectives in Marine Biology" a few years ago the distinguished oceanographer Dr. Alfred C. Redfield gave a paper with the rather provocative title: "On the inadequacy of experiments in marine biology" (Redfield, 1958). Although admitting the great value of contributions from physiologists, Redfield concluded that "the greater need at the moment is more knowledge of the phenomena to be explained." One can follow Redfield a long way in his argument for intensifying the study of conditions in the sea in order to obtain a more precise description of the qualitative and quantitative distribution of populations as related to environmental factors. However, marine biologists working on field ecological studies may not in general wholeheartedly agree with his conclusion. It may not be wrong to say that there is a general feeling among marine ecologists that precisely the combination of field work and laboratory experiments is essential to progress in the biological field of oceanography. Another question is whether this feeling is sustained by the results, which have so far been achieved through experiments. The general title of this paper is so wide that it will be necessary to illustrate the experimental approach with the use of cultures by examples and these have been chosen from my own field of marine phytoplankton. I would like to make it clear that I have chosen to abstain from dealing with the more general studies of, say, plant physiology, which undoubtedly are of the greatest value for the

study of marine phytoplankton in general. In this way the scope of the following discussion will mainly be a consideration of experiments dealing with specific differences among the members of the marine phytoplankton in their reaction to the environment.

Field observations have revealed a variety of patterns in horizontal and vertical distribution, and pronounced annual cycles of species, leading to a succession of plankton societies. It is characteristic of the marine phytoplankton that the details in this succession may change from year to year in the same habitat. An analysis of these striking events requires knowledge of the reaction of each species or group of species to the environmental conditions.

The reason for my taking this opportunity to review the autecological aspect especially is not that there are many spectacular results to present. On the contrary, the scarcity of available experimental data forces me to stress mainly the need for an intensification of effort, in order to establish a harmonic progress in the study of the producers in the sea.

Let us first review briefly what information studies on cultures have afforded as to the influence upon various marine phytoplankton species of such environmental factors as salinity, temperature, light, and inorganic nutrients, which are subject to routine recording in oceanographic investigations. Salinity and temperature are generally accepted as important for the distribution of species, while light and inorganic nutrients are recognized as controlling factors for growth of the population in general. As the first of these classical ecological factors we may consider salinity.

Salinity

For the diatoms, which form such an important part of the planktonic vegetation, even in tropical waters (Hasle, 1959), the only records on how salinity affects growth are from Kain and Fogg's (1958) experiments on *Asterionella japonica* (Fig. 1). They indicate an optimum at a salinity of 30‰ and no growth at 15‰ and below. In Danish waters, with a wide range in salinity, the

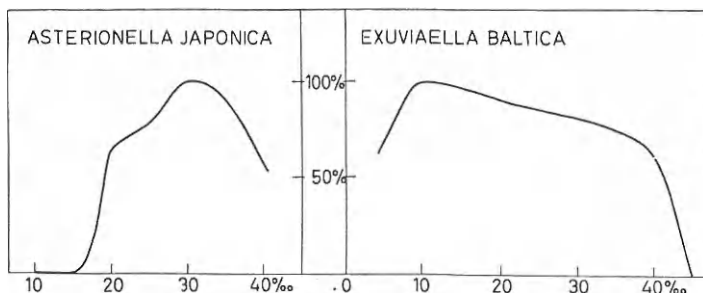


Fig. 1. Growth at different salinities as percentage of maximal growth rate (Kain and Fogg, 1958; Braarud, 1951).

samples with *Asterionella* showed a mean of 31.5‰ (Ostenfeld, 1913) and, likewise, its general occurrence in the Atlantic and the Mediterranean at salinities above 35‰ and its absence in brackish waters accord well with the experimental findings.

Cultures of dinoflagellates isolated from the Oslofjord show somewhat different curves for the salinity effect upon growth. Let us first consider *Exuviaella baltica* (Fig. 1). The growth rate is remarkably high within the whole range from 10‰ to 40‰, with a slight indication of an optimum around 10‰. These results are in agreement with field records of its abundant occurrence in waters of widely different character. It occurs in the brackish waters of the Baltic, in the waters of varied salinity around Denmark, in the North Sea and the coastal waters along Norway, in the oceanic parts of the Norwegian Sea and the North Atlantic, in the Barents Sea, and even in polar waters along the east coast of Greenland. Obviously salinity does not restrict its distribution, except in marine waters of extremely low salinities.

If we consider the other dinoflagellates from the Oslofjord area (Fig. 2), we may first point out that, like *Exuviaella baltica*, these also have optima at salinities below 30‰, namely between 15‰ and 20‰, values which coincide with those for the surface waters in the Oslofjord in summer, when these species occur in greatest abundance. The main difference between the species is found in their response to low salinities.

Peridinium triquetrum, is characterized by fairly good growth

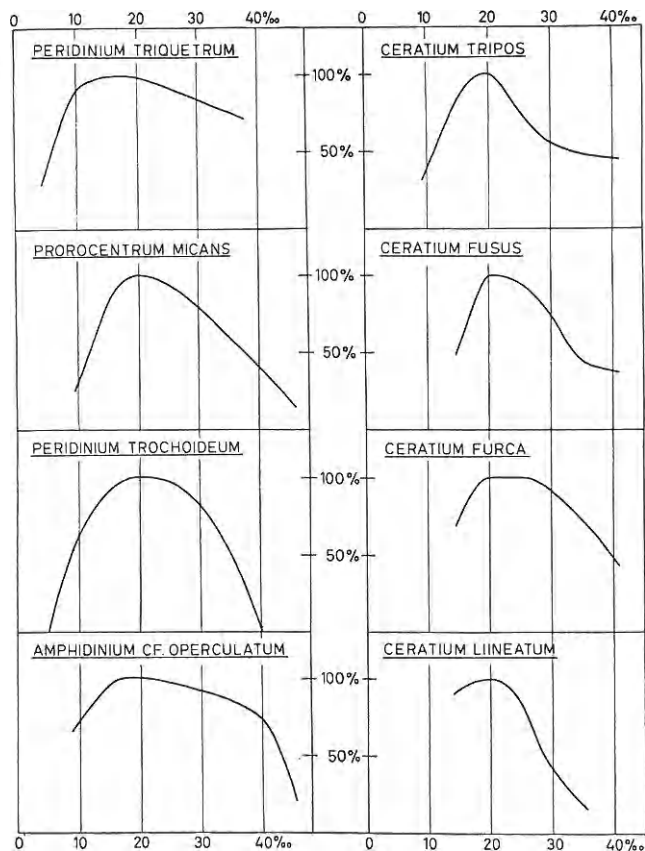


Fig. 2. Dinoflagellates: growth at different salinities, as percentage of maximal growth rate (Braarud 1951; Braarud and Rossavik, 1951; Nordli, 1957).

even at 5‰ (Braarud and Pappas, 1951). This explains how this species is able to thrive as far into the Baltic as in the harbor of Helsingfors, where the salinity, according to Valikangas (1926), may vary between 3.5‰ and 6‰. The *Ceratium* species also show differences in this respect, *C. tripos* being the only one in Nordli's experiments (1957) which reproduced at 10‰ (Fig. 2). This species is known to penetrate farther into the Baltic than the other species of this genus, although it does not go as far in as *Peridinium triquetrum*. This feature in its distribution finds a reasonable explanation in the response observed in the salinity experiments.

For the dinoflagellate species *Exuviaella baltica*, *Peridinium triquetrum*, and *Ceratium tripos*, the salinity experiments have thus given an explanation of characteristic features in their distribution in habitats of relatively low salinity. In the other species investigated, there is an overall agreement between the results obtained in the experiments and the known distribution of the species. This fact indicates that experiments on clone cultures from one locality may give results which are applicable to the population of the species within a larger area.

Beforehand one might have reason to doubt whether this would be the case. In experimental work of this kind one may question whether clones or strains are constant in their response to environmental conditions; whether a single clone is representative of a local population, and one may even ask if there exist geographical races, especially in species with a very wide distribution. Only a very few observations are available for elucidating these points, which are of obvious importance when trying to apply experimental results to the understanding of what happens in the sea.

In Fig. 3 are shown graphs from salinity experiments on the same clone of *Peridinium trochoideum* at different temperature conditions and with media made up of different batches of sea water. It will be seen that the general features of these curves are similar: an optimum appearing at about 20‰, while reproduction at higher salinities differs somewhat. Two clones of *Prorocentrum micans*, both from the Oslofjord, but isolated at different times, also show

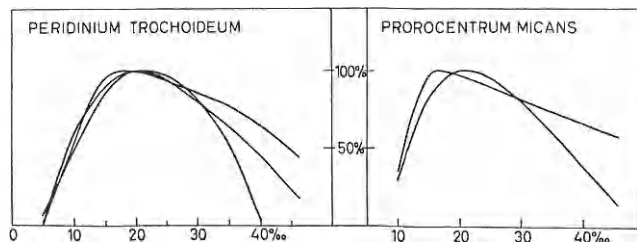


Fig. 3. Growth at different salinities as percentage of maximal growth rate. *Left*, experiments on the same strain from Oslofjord; *right*, experiments on Oslofjord strains from different years (Braarud, 1951).

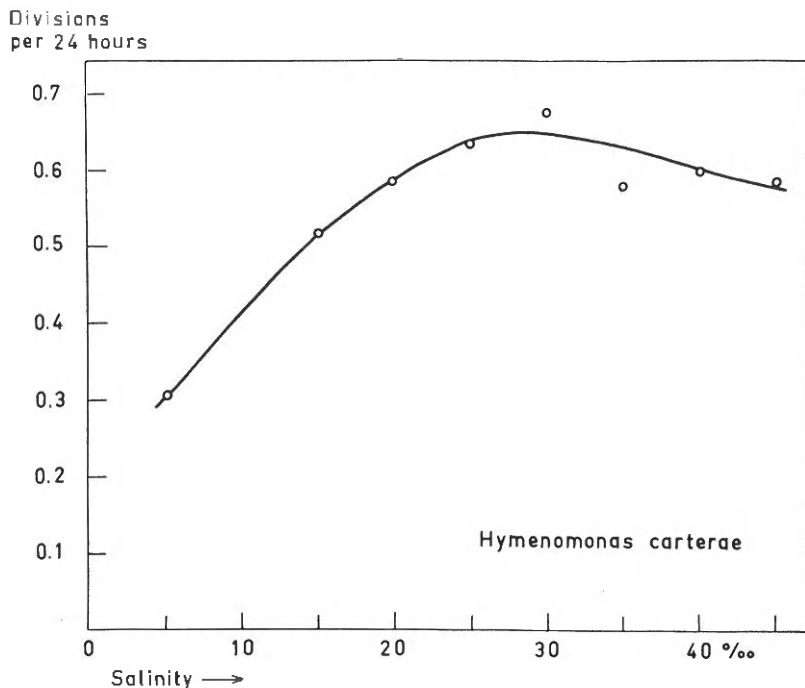


Fig. 4. *Hymenomonas carterae*: growth rate at different salinities (Braarud, 1951).

essentially similar features in their response to salinity: Low growth rates at 10‰ and a quick rise to 15–20‰, with a slow decline when salinity rises to 40‰.

For clones of the same species originating in widely separated localities, there is one observation, again of *Peridinium trochoideum*. The salinity-growth relationship has been studied in clones from the Oslofjord, where the salinity during its abundant occurrence in summer is about 20‰, and from Golfo di Napoli, where salinity is as high as 37‰. These clones gave practically identical curves with an optimum of salinity as low as 20‰. These last results indicate a uniform salinity response in populations inhabiting waters with widely different salinity conditions, thus giving no hint of geographical races in this species, which has a worldwide distribution.

Turning now to the third important group in the marine phytoplankton, the coccolithophorids, there are only two species whose salinity-growth relationship has been studied: *Hymenomonas carterae*, originally referred to *Syracosphaera* (Braarud, 1951), and *Coccolithus huxleyi* (Mjaaland, 1956). *Hymenomonas carterae* is, as far as we know, an inshore species. The clone used in the experiments was isolated from an artificial oyster pond where the salinity during its occurrence varied between 17‰ and 30‰. In culture it showed very good growth between 15‰ and 45‰, but even at 5‰, growth was fair (Fig. 4). Obviously salinity is not an important environmental factor for this species, which has only been observed in abundance in littoral pools (Carter, 1938; Braarud and Fagerland, 1946) where salinity is apt to show great variation.

The other coccolithophorid, *Coccolithus huxleyi*, showed good growth between 20‰ and 45‰, while it was also able to grow at 15‰ (Fig. 5). This is in accordance with its worldwide distribution in oceanic waters of salinities around 35‰ and in coastal waters of northern Europe as well, where it may occur in extremely dense populations at lower salinities of between 15‰ and 20‰ (Lohmann, 1908; Birkenes and Braarud, 1952). The experimental results indicate that it requires too high a salinity to be able to populate truly brackish waters.

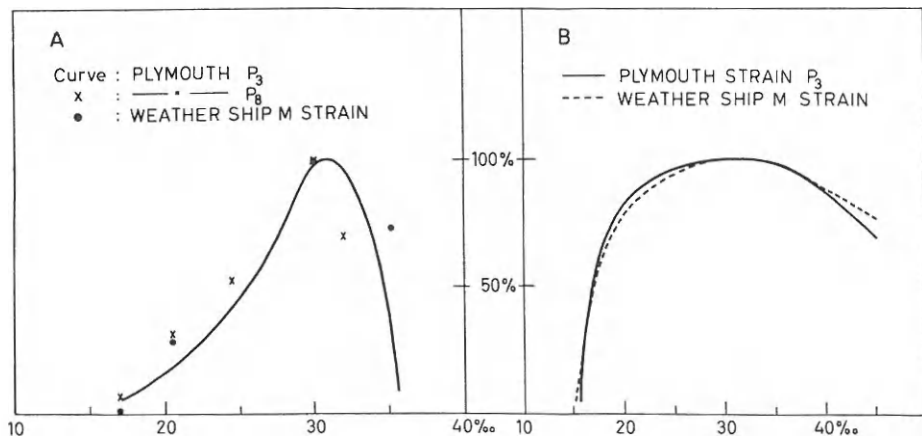


Fig. 5. Growth of *Coccolithus huxleyi* at different temperatures (A) and salinities (B) as percentage of maximal growth rate (Mjaaland, 1956).

Looking back upon these scattered observations of growth response in marine phytoplankton to the salinity of the environment, it may be stated that, so far as these go, they justify confidence that information from experimental results may be applied successfully in interpreting records from surveys at sea. The consistent results for different clones of dinoflagellates from the same locality, or from widely separated localities, indicate that even with the use of a clone from only one locality, the results may be applied for populations of a large area. However, when we turn to the temperature experiments, we soon see that the picture is more confused.

Temperature

Since the first biogeographical surveys of marine phytoplankton, temperature has been regarded as an essential ecological factor for the geographical distribution of each species. Terms such as arctic, boreal, temperate, and tropical species are generally used with the implication of a decisive temperature effect upon growth and survival. In trying to explain the succession of diatom species in north European coastal waters during the spring maximum and the following decline, temperature has also been suggested as an important factor. Attempts at verifying the validity of these assumptions through experiments are, however, few.

In Table I data have been gathered from field observations and from experiments for the few planktonic diatoms which have been studied as to the effect of temperature upon growth. Schreiber

TABLE I. Temperature-Growth Relationship in Marine Plankton Diatoms^a

Species	Temperature during Periods of Abundance	Optimal Temperature in Culture
<i>Biddulphia aurita</i>	1°C	(5°C)
<i>Biddulphia sinensis</i>	13°C	(16°C)
<i>Asterionella japonica</i>	8°C (Gran)	20–25°C
	Above 20°C (Danish invest.)	
<i>Thalassiosira nordenskiöldi</i>	2–3°C	Excellent growth at 10–11°C

^a According to Ostenfeld, 1913; Schreiber, 1927; Gran, 1929; Braarud, 1937; Grøntved, 1949.

(1927) found that the winter species *Biddulphia aurita* showed far better growth at low temperature than at ordinary room temperature, whereas the summer species *Biddulphia sinensis* reproduced slowly at 5° and 9°C, but showed good growth at 16°C. Schreiber concluded that this difference in temperature response gives an explanation of the different seasonal occurrence of the two *Biddulphia* species in the North Sea.

On the other hand, recent experiments on *Asterionella japonica* by Kain and Fogg (1958) gave results which are at variance with those obtained by some of the field observations. Gran (1929) reviewed the available field observations and concluded that *Asterionella*, judged from its distribution, has a temperature optimum at about 8°C, and that it is also abundant in waters of lower temperature, a conclusion which was supported by subsequent records from the North Sea area by Braarud *et al.* (1953). Kain and Fogg (1958) observed, however, a definite temperature optimum at 20–25°C and no growth below 10°C in a nonbacteria-free culture (Fig. 6). The picture is becoming still more complicated by the observations from Danish inshore waters by Grøntved (1949) demonstrating abundant occurrence of *Asterionella* at a temperature of 20–25°C. It becomes evident that neither the conclusions drawn from field observations by Gran (1929) nor the experimental results by Kain and Fogg (1958) provide an adequate characterization of the temperature response of *Asterionella*.

Another discordance between the sea temperature during periods of abundant occurrence and its growth in culture was observed by Braarud (1937) for *Thalassiosira nordenskiöldi*, a boreal neritic diatom species which also occurs in polar waters. Ostenfeld (1913) gives a mean temperature for its occurrence in Danish waters of about 2°C, a temperature which is similar to that found by Gran and Braarud (1935) for waters showing the greatest abundance of this species in the Fundy region and likewise, by Ramsfjell (1954) and Paasche (1959) for the Norwegian Sea. In culture, however, Braarud found that it showed excellent growth also at a temperature as high as 10–12°C, with growth rates which presumably are near to the maximal ones for the species.

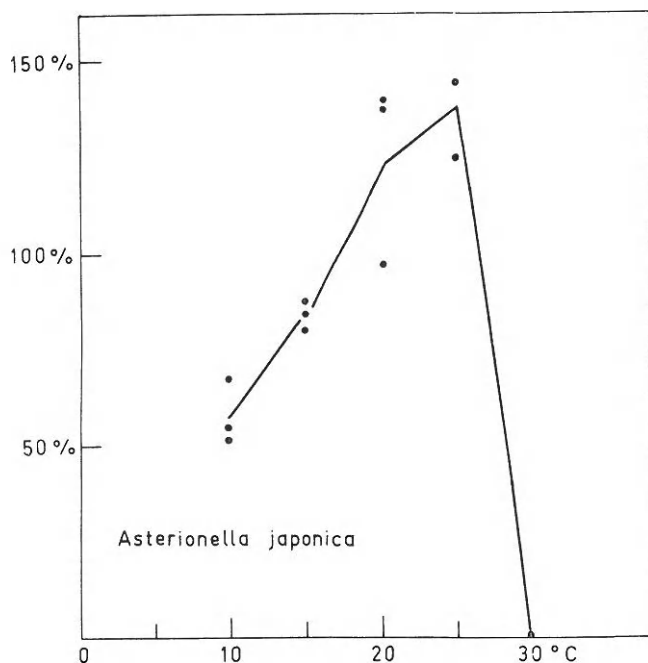


Fig. 6. *Asterionella japonica*: Growth at different temperatures as percentage of controls (Kain and Fogg, 1958).

Before discussing how the results for *Asterionella japonica* and *Thalassiosira nordenskiöldi* may be interpreted, we may look at observations of representatives for the two other main groups.

For dinoflagellates the growth-temperature relationship of a number of species has been studied in nonbacteria-free strains from the Oslofjord. The results are presented in Fig. 7. In all these cultures optimum was observed in the region of 15–20°C, which is the temperature range for summer, when these species, with the exception of *C. lineatum*, are found in greatest abundance. *Ceratium lineatum*, which Nordli (1957) in his experiments found to have an optimum at 20°C also, has its main occurrence in the Oslofjord at seasons when the temperature is lower. This species shows, however, good growth at lower temperatures as well and other ecological factors, such as the nutrient supply, may be responsible for its prevalence in seasons other than summer. The

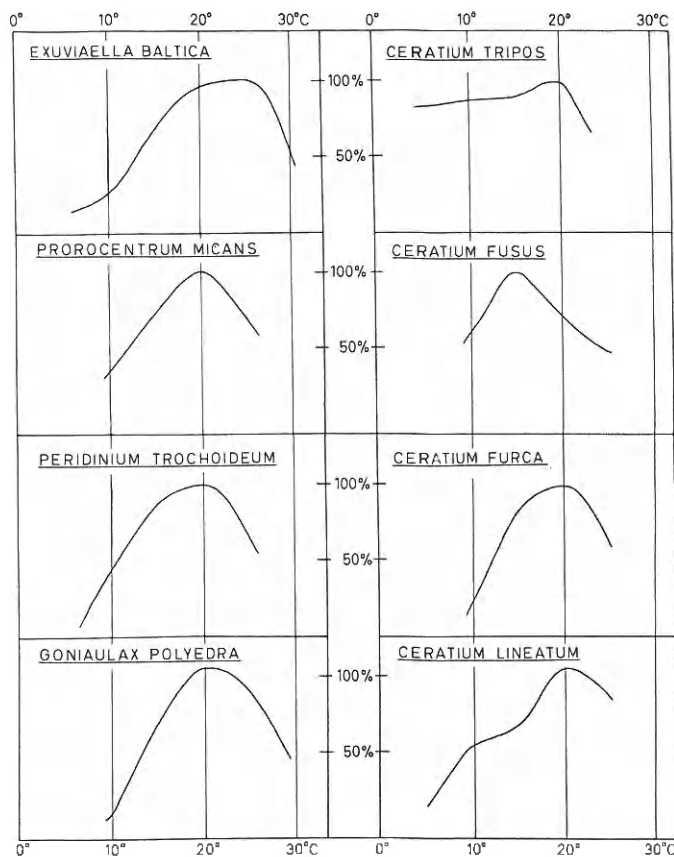


Fig. 7. Dinoflagellates: growth at different temperatures as percentage of maximal growth rate (Bakken, unpublished; Nordli, 1957).

relatively high growth rate of *Ceratium tripos* at low temperatures, as compared with the other ceratia studied by Nordli, gives a reasonable explanation of the predominance of this species in the cold season in the Oslofjord area, as compared with other species of the genus such as *Ceratium furca* and *fusus*.

In recent experiments on a strain of *Peridinium trochoideum* from Golfo di Napoli, the temperature optimum was found to be as high as 25–30°C (Fig. 8), while the Oslofjord strain showed an optimum at 20°C as did the other dinoflagellates from the Oslofjord area (Fig. 7).

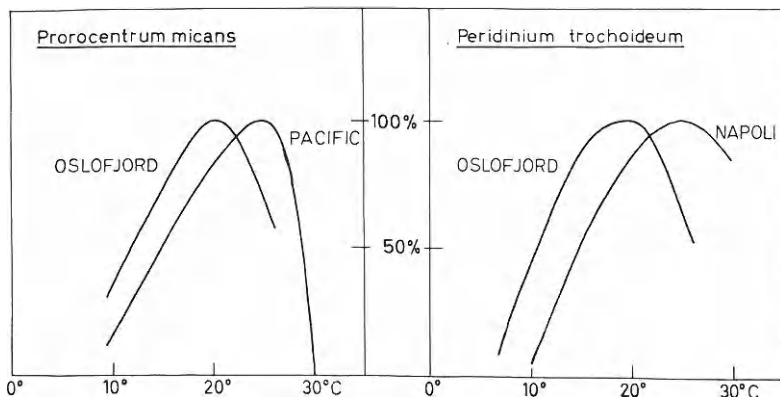


Fig. 8. Temperature response in strains from different geographical regions (left, Barker, 1935, and Bakken, unpublished; right, Bakken, unpublished, and Braarud, unpublished).

A similar displacement of the optimum is known from temperature experiments on *Prorocentrum micans*. The Oslofjord strain of this species showed a definite optimum at 20°C, whereas Barker (1935) in experiments with cultures from the coast of California observed an optimum at 25°C (Fig. 7).

In the coccolithophorid *Coccolithus huxleyi* Mjaaland (1956) observed a temperature optimum at about 20°C and growth at as low a temperature as 7°C (Fig. 5). This reaction is in fair agreement with observations from surveys, although these indicate that growth may even take place at a considerably lower temperature.

For the available temperature experiments the comparison with field observations gives a less coherent picture than the salinity experiments. There are examples of a general agreement between the experimental results and the field data, as for the dinoflagellates from the Oslofjord, and there are instances of pronounced disagreement as for *Asterionella japonica* and *Thalassiosira nordenskiöldi*. Two explanations may be suggested for these discrepancies and both may be involved: that the experimental technique has been unsatisfactory and given erratic results, or, that the deductions from biogeographical data are erroneous and have blurred the ecological picture for the species in question, as sug-

gested by Braarud (1937) in the case of *Thalassiosira nordenskiöldi*.

The main objection to the technique used may be that the cultures were not bacteria-free. In such a case two effects may be responsible for the different growth rates of a culture at different temperatures: (1) the response of the alga to the temperature factor, the response which we are looking for, and (2) the response of the bacterial population in the culture which may, indirectly, influence the rate of growth of the algal population. As long as we have no parallel temperature experiments with and without bacteria as associates, we are unable to judge how important the latter effect may be, but the recent discovery of the vitamin requirements of the plankton algae lends some support to such a view. It would not seem unreasonable to suggest that the difference between the temperature optima observed in the experiments with strains of *Peridinium trochoideum* and *Prorocentrum micans* from widely separated localities might be due to the difference in the bacterial populations of the two cultures, since the latter may presumably provide vitamins necessary for growth.

The other alternative, that temperature is not a decisive environmental factor for the abundance of a species such as *Thalassiosira nordenskiöldi* at low temperature and its absence at higher temperature, as indicated by the experiments, finds some support in the fact that the rise in temperature in spring in northern Atlantic waters coincides with a decline in the supply of inorganic nutrients. Species demanding fairly high concentration of nutrients, therefore, might show a similar seasonal occurrence as a cold water species. Only new experiments with bacteria-free cultures can give us a clearer picture of the actual temperature effect upon growth.

From the experiments on the salinity and temperature factors it may be concluded that the tolerance of the various species to extreme conditions should be studied with special care.

The two remaining environmental factors of this group, light and nutrient supply, can be dealt with quickly as very few observations are available for a discussion of specific differences. Photosynthesis experiments with cultures of diatoms suspended

at different depths were carried out by Marshall and Orr (1928) and by Jenkin (1937). These classic investigations demonstrated clearly the important feature that, in the diatoms which they used in their experiments, an inhibition of photosynthesis occurred near the surface, the depth for the photosynthesis optimum varying according to time of day and season. Marshall and Orr found that the winter species *Coscinosira polychorda* and a summer species of *Chaetoceros* had the same optimum light intensity but that inhibition at the surface was more pronounced in the *Chaetoceros* species. The latter result was unexpected, and the authors suggest that it might be due to the fact that the *Chaetoceros* species had been grown in weak light. Jenkin worked on the diatom *Coscinodiscus excentricus* and to a lesser extent on *Biddulphia regia*, which are both winter forms. They were found to have similar optimum light intensities.

Jenkin concluded that the greatest scope for future work would seem to lie in the laboratory, but during the twenty years which have elapsed since her paper was published, only incidental observations of this kind have been made. Mjaaland (1956) observed excellent growth in the coccolithophorid *Coccolithus huxleyi* even at as strong light as 50,000 lux while Nordli (1957) in his experiments on ceratia, observed an optimum between 2,500 and 5,000 lux. He concluded that his results were at variance with the vertical distribution pattern of these species. Lanskaja and Sivkov (1950), who studied growth of five diatoms, remark that there is reason to believe that photosynthesis and also growth may differ according to the state of the cells and their previous history, a view also held by Marshall and Orr (1928). This is in accordance with the behavior of other algae, and may explain the extremely low optimum intensities observed by Schreiber (1927) in the case of the two species of *Biddulphia*.

The general effect of light upon phytoplankton photosynthesis and growth seems to be well established by studies reviewed by Steemann Nielsen (1958b), and by Steemann Nielsen and Jensen (1957), but it is obvious that too few investigations of the light response of the various species are available for a comparison with their distribution in the sea. Field observations indicate

that in this respect also we may find important specific differences, but much basic work is required in order to obtain a satisfactory method for further studies.

The possibility of different responses to varying concentrations of inorganic nutrients was brought into the spotlight by Rodhe (1948) through his observations of extremely low maximal values for various freshwater species. For the truly marine forms experiments are not available to judge whether this ecological factor is responsible for exceptional distribution patterns in certain species. So far, the experimental approach in studying the environmental influences on marine phytoplankton has been for orientation purposes. In future special attention should be paid also to the interaction of the various factors.

The technique of the experiments has been simple. The cultures have not been bacteria-free. The media have been enriched sea waters, with or without soil extract as a supplement, and consequently not well defined. As long as this was the case, problems of more special nutritional requirements could not be approached. These defects have been obvious to workers in the field and may be partly responsible for the restricted nature of the activities.

Since the pioneer research on cultures of marine phytoplankton initiated by Miquel (1890-93) and especially by Allen in Plymouth more than half a century ago, a great number of attempts have been made to introduce synthetic media by using artificial sea water. Allen himself, partly in collaboration with Nelson, made experiments of this kind (Allen and Nelson, 1910; Allen, 1914) and made the important observation that only with the addition of small amounts of natural sea water could satisfactory growth be obtained. Subsequent work proved that in most cases growth in artificial sea water was not as good as in Allen or Schreiber sea water with soil extract, or that no growth at all took place. The importance of the organic components of natural sea water for diatom growth was demonstrated by Harvey (1939), but it was not till the general occurrence of auxotrophy in marine phytoplankton had been discovered that the basis was laid for introducing well-defined media of a satisfactory composition. Through the contributions by Provasoli, Hutner, and their collaborators in

New York, by Droop in Millport, and Sweeney at La Jolla (for literature, see Droop, 1957) information has been obtained on the vitamin requirements of representatives for marine diatoms, dinoflagellates, and chrysophyceans, especially as to vitamin B₁ and B₁₂. These results have been reached by means of an improved technique which we shall consider before presenting some of the findings of their research into the necessity of organic compounds for growth.

The new technique is the result mainly of the work by Provasoli and his collaborators (see Provasoli *et al.*, 1957) on the composition of media, and of the success of various workers in establishing bacteria-free cultures. Compared with the older synthetic media the new ones are characterized by the following improvements: (1) a uniform and satisfactory supply of micronutrients by means of chelating agents as EDTA (ethylenediamine tetraacetic acid), (2) buffering of the medium by means of a compound which is not used during the growth of the alga, as TRIS (hydroxymethylaminomethane), and (3) addition of defined quantities of organic compounds required by the alga, as vitamin B₁ and B₁₂, which were previously supplied by the natural sea water and the soil extract (Sweeney, 1954).

The use of these compounds has made it possible not only to obtain satisfactory growth in synthetic media with a general composition similar to that of sea water but also to make essential changes in the ionic composition of the medium for a study of the relative importance of the various components of sea water, as made by Provasoli *et al.* (1954). Droop (1958b) has used this technique in a study of euryhaline species from littoral pools and of the pelagic diatom *Skeletonema costatum* and has drawn the tentative conclusion that the different salinity response of the various species is due mainly to their special requirements for the concentration of sodium ions in the medium.

One of the main drawbacks with the Allen technique was the presence of bacteria and colorless flagellates in the persistent cultures. Schreiber (1927) devised a special apparatus for washing diatoms in order to get rid of bacteria. Jenkin (1937) used a treatment with iodine, suggested by Allen, for destroying at least the

major part of the bacterial population, but did not obtain truly pure cultures. During recent years bacteria-free cultures of several species of marine diatoms and flagellates have been obtained. Two methods have been used: (1) simple washing with sterile medium and (2) treatment with antibiotics, with minor variations in the procedure (for literature, see Spencer, 1952; Droop, 1954). If these methods are combined with the use of a varied selection of media, there should now, as indicated by Droop, be a far greater chance of success in establishing bacteria-free cultures of the autotrophic members of the marine plankton, although obstacles may still be encountered (Kain and Fogg, 1958). Special efforts ought to be made to improve still further the isolation technique.

We may now return to the results which have been obtained by the use of this new technique. Droop (1957) has summarized the results on the vitamin requirement of marine phytoplankton species up to 1957. Table II shows that the four chrysophyceans which were studied in bacteria-free cultures have the same requirements for vitamin B₁ and B₁₂, while the two diatoms, *Phaeodactylum tricornutum* and *Skeletonema costatum*, differ. *Phaeodactylum* requires neither of them, whereas *Skeletonema*

TABLE II. Vitamin Requirements in Bacteria-Free Cultures (Droop, 1958a)

	B ₁₂	Thiamine	Portion of Thiamine Required
Chrysophyceans			
<i>Monochrysis lutheri</i>	+	+	} Pyrimidine
<i>Prymnesium parva</i>	+	+	
<i>Syracosphaera elongata</i>	+	+	
<i>Microglona arenicola</i>	+	+	
Diatoms			
<i>Phaeodactylum tricornutum</i>	—	—	
<i>Skeletonema costatum</i>	+	—	
Dinoflagellates			
<i>Oxyrrhis marina</i>	+	+	Thiazole
<i>Glenodinium foliaceum</i>	+	—	
<i>Peridinium trochoideum</i>	+	—	

requires B_{12} . One of the dinoflagellates, the nonphotosynthetic *Oxyrrhis marina*, requires both, whereas the two photosynthetic species probably have no need for B_1 in the medium. The chrysophyceans require the pyrimidine component of thiamine, while the dinoflagellate *Oxyrrhis* uses the thiazole component.

The possible ecological implications of these results we shall leave for a brief consideration later, but there is another principal feature which may be stressed here. Although only a few representatives of the various taxonomic groups have been investigated, the results indicate a diversity in the requirements of various species within the same taxonomic group, as shown for the diatoms and the dinoflagellates. This is still more clearly demonstrated by the response to various compounds of the B_{12} group as presented by Provasoli (1958). As will be seen from Table III, there are in chrysomonads and in dinoflagellates differences as to their ability to utilize the various cobalamins, two types being represented among the chrysomonads and two within the dinoflagellates, while the diatoms add to this diversity.

These results make it evident that generalizations of results obtained for this feature of the metabolic pattern, would be unwarranted. They may also be taken as a sign that great variations may occur not only in this aspect but also in the general response of the various species of a taxonomic group to the composition of the medium and other environmental factors. This was also indicated by the experiments on the salinity factor which have been referred to previously. What the situation will be if we consider ecological groups, established by field observations, is a question that cannot be answered until more species have been examined.

Provasoli and Pintner (1953), Provasoli (1958), and Droop (1957) have discussed the possible importance of the auxotrophic demands of the various species for their occurrence in the sea. Undoubtedly interesting ecological implications suggest themselves while the available information on the occurrence of vitamins in the sea continues to be rather scanty (Cowley, 1956), so it would seem premature to draw definite conclusions. The work on vitamin requirements is, however, of paramount importance for experimental studies on cultures. In addition to the other im-

TABLE III. Specificity of Marine Organisms toward Cobalamins (Provasoli, 1958)

Organisms	B ₁₂ (5,6-dimethyl-benzimidazole)	5,6-Di-chloro-benzimidazole analog	Benzimidazole analog	B ₁₂ in (Factor I)	Factor A (2-Methyladenine)	Factor H (2-Methyl-xanthine)	Pseudo B ₁₂ (Adenine)	Factor B (Nucleotide)
<i>Chrysomonads</i>								
<i>Prymnesium parvum</i>	+	+	+	+	○	○	○	○
<i>Microglona arenicola</i>	+	+	+	+	○	○	○	○
<i>Syracosphaera elongata</i>	+	+	+	+	○	○	○	○
<i>Isochrysis galbana</i>	+	+	+	+	○	○	○	○
<i>Monochrysis lutheri</i>	+	+	+	+	+	+	+	○
<i>Dinoflagellates</i>								
<i>Gyrodinium californicum</i>	+	+	+	+	○	○	○	○
<i>Gyrodinium resplendens</i>	+	+	+	+	○	○	○	○
<i>Gyrodinium uncalanum</i>	+	+	+	+	○	○	○	○
<i>Amphidinium klebsii</i>	+	+	+	+	+	+	○	○
<i>Amphidinium rhynchocephalum</i>	+	+	+	+	+	+	○	○
<i>Diatoms</i>								
<i>Skeletonema costatum</i>	+	+	+	+	+	+	+	+
<i>Amphora perpusilla</i>	+	+	+	+	+	+	+	+

Note. + = active; ○ = inactive.

provements of the media, the knowledge of the need for these organic compounds and the success with the isolation of bacteria-free cultures seem to promise that a new era has been initiated in experimental studies of the autecology of the marine phytoplankton.

There are also other indications that a more satisfactory basis for such work can now be established. I refer to recent work on the life cycle of the plankton algae. The species, which would be most suitable for culture work and studies on the influence of environmental conditions on their occurrence, are those which reproduce by binary fission, without any complications in the form of sexual reproduction or cyst formation. Among the more important groups of plankton algae, the dinoflagellates seem to be closest to this ideal. None of the marine brown dinoflagellates which have been cultured, representing species of *Amphidinium*, *Ceratium*, *Exuviaella*, *Goniaulax*, *Gymnodinium*, *Gyrodinium*, *Peridinium*, *Prorocentrum*, and *Protoceratium*, has shown any sign of sexual reproduction. Some of them form cysts when the cultures become too dense or old, but there are no difficulties in keeping the cultures in active growth without cyst formation.

The diatoms, on the other hand, are not so easily handled in culture work. Their special mode of cell division, whereby one of the daughter cells becomes smaller than the other, leads to a variation in cell size within a population. Transitions from narrow to broad cells take place through auxospores, in connection with sexual reproduction. In species where auxospore formation takes place readily, the average cell size may not vary excessively, while in other species, which form auxospores at longer intervals, populations with very different values for the average cell size may occur both in nature and in culture. As the growth rate is likely to vary with cell size, more extensive experiments must be carried out in order to obtain results which are applicable for interpretation of field observations. For the culture work an intimate knowledge of the life cycle of the species in question and the conditions of sexual reproduction are therefore of great value. Through the work of von Stosch (1954) the methods for life cycle studies on centric diatoms, which are predominant in the sea, have been

demonstrated, and this field calls for experimental work on a large scale. Field observations indicate that there is considerable variation between the species as to the frequency of auxospore formation. Detailed information on this point, required for a satisfactory handling of diatom cultures in autecological work, can be obtained only through laboratory experiments.

The third large group of plankton algae, the coccolithophorids, has also, one may say rather unexpectedly, disclosed a complicated life cycle. In *Coccolithus fragilis* Bernard (1939) has suggested a tentative life cycle, which includes the regular occurrence of palmella stages in the sea. Quite recently Parke (1959) has been able to show experimentally that the species *Crystallolithus hyalinus* represents the motile stage of the well-known *Coccolithus pelagicus*, which, according to Parke, has the character of a cyst. This is an observation which gives quite a new viewpoint to the understanding of distribution related to environment in these forms. Observations on *Coccolithus huxleyi*, the commonest of all marine coccolithophorids, indicate that problems are also in store concerning the life cycle of this species.

This type of experimental work has been dealt with so extensively here because it is essential both in direct interpretation of the observations from the sea and in the introduction of a technique whereby uniform material for culture work may be obtained. The recent achievements give promise of obtaining very important information through continued studies within this field.

Looking back upon the work which has been done on cultures of marine phytoplankton, we notice that the experimental results have been related to only a few of the many problems facing a field ecologist. For many reasons it was natural to start with environmental factors such as salinity and temperature, which had been used so extensively for interpretation of specific differences in distribution. A basic knowledge of the influences of these factors would also seem to be a prerequisite for a closer study of the finer details in distribution pattern and seasonal occurrence.

As activities have been so restricted and, unfortunately, a great part of the experimental work has been made on species which are not observed regularly in the sea, such as *Phaeodactylum*

tricornutum, the former *Nitzschia closterium* f. *minutissima*, there is a host of unsolved problems calling for studies by means of experiments with the use of cultures. The mention of a few problems may be allowed.

The annual cycle of the phytoplankton, which has such characteristic features in higher latitudes, has been investigated in all-year surveys and, in many cases, the general trend has been found to relate well with changes in the supply of nutrients, stability conditions, and light supply, but we cannot yet give any substantiated explanation of the succession of species. Several theories have been advanced, but these have a common lack of background knowledge regarding the actual behavior of the organisms in question. We have already seen how doubtful the information is on the temperature response on the few species which have been studied. The situation is no better in the case of the mutual effect of species growing together, for instance by means of ectocrine substances. This factor has been suggested as playing a part in producing succession, but this has still to be proved. The different rate of growth in the various members of a society is obviously of prime importance for their relative abundance, but actual knowledge of the rate of reproduction in diatoms, dinoflagellates, and coccolithophorids is so fragmentary that it cannot be used for estimating with any certainty the effect of this feature in individual cases.

Extreme mass occurrence of certain species, as for instance in red tides, represents an interesting and spectacular example of societies presumably resulting from hitherto obscure conditions favoring growth in certain species. These are normally members of societies of a more balanced composition.

Form variation is observed in every species. In some cases there seems to be a phenotypic variation which has been tentatively related to the influence of temperature, salinity, nutrient salt concentration, and day length. Experimentally little has been done in this field. For *Ceratia*, Nordli (1957) observed variation in size with changes in temperature and, in the case of rather abnormal forms, he found their occurrence to be related to an ample supply of nutrients and the absence of a marked day-night rhythm. How-

ever, in general it may be stated that the actual effect of the environmental factors upon form has not been analyzed.

The conditions which induce sexual reproduction and lead to auxospore formation in the various species of diatoms are also obscure. Likewise, are the conditions stimulating cyst formation in diatoms, dinoflagellates, and coccolithophorids. We know that these processes occur regularly in some species, whereas in others they seem to be rarer, presumably occurring as a reaction to rather special environmental conditions. Germination of resting spores has never been studied in cultures, except for the rather unusual type observed by Gross (1940) in *Ditylum brightwelli*, although it would be of great interest to obtain information on this process in order to further understanding of the annual cycle of many neritic species.

One of the basic features of behavior in plankton diatoms, their ability to maintain populations in the surface layers even during periods of poor growth conditions, is still one of the dark points. The various species seem to react differently in this case also, as some of them maintain small populations in the euphotic layer throughout the year, while others have brief periods of abundance and then disappear. The field observations call for experimental studies, both to investigate further the interesting theory of Gross and Zeuthen (1948) that flotation is due to the maintenance of a relatively high concentration of monovalent ions in the cell sap and also to explain the specific differences in behavior indicated by the field observations.

The effect of submarine light upon the phototaxis of flagellates has been subjected to interesting physiological studies by Halldal (1958), but the specific reactions of most species have yet to be described on the basis of laboratory studies. The observations at sea have shown that migrations take place, and have indicated different reaction patterns for the various species (Hasle, 1950), but experimentally very little has been done here. These are a few of the classical problems that must be solved by experimental means, and they are involved in most of the attempts at understanding the results produced by field surveys. The studies of organic production in the sea by means of Steemann Nielsen's

C¹⁴ method (1952, 1958a), which have given such valuable results, have again raised many problems related to the actual behavior of the species which are responsible for the observed production.

These examples may suffice to indicate the variety of unsolved problems that are suitable for experimental studies. In spite of the shortcomings in methods which still adhere to the field ecological surveys of marine phytoplankton, it seems obvious, as far as this branch of marine biology is concerned, that Dr. Redfield's warning against too one-sided an experimental approach does not apply at all. The experimental studies have by no means kept pace with the observations of populations in the sea. On the other hand, the little work which has been done on phytoplankton cultures has demonstrated how essential a background of field observations is for a sound evaluation of experimental results. One need refer only to the temperature experiments, which illustrated so clearly the necessity of checking results obtained in the laboratory with the actual distribution pattern of the species in the sea. On the whole, the experience gained from experimental studies supports the generally adopted view that research on environmental influence should be organized in such a way as to establish a close collaboration between workers with a first-hand knowledge of field surveys and skilled experimentators.

With regard to the special branch discussed here, the time seems ripe for an extension of research in the laboratory. The new techniques developed recently should be employed and developed further to exploit more effectively the wealth of observations accumulated through field ecological studies. In view of the fact that the marine vegetation is responsible for at least as large an annual production of organic matter as that of all the terrestrial plants, there seems to be no reason why such an expansion should be made at the expense of work of the survey type. The present efforts in this field seem to be quite out of proportion to the role which these marine plants play in the organic cycle as a whole.

REFERENCES

- Allen, E. J. 1914. On the culture of the plankton diatom *Thalassiosira gravida* Cleve in artificial sea-water. *J. Marine Biol. Assoc. United Kingdom*, 10, 417-439.

- Allen, E. J., and E. W. Nelson. 1910. On the artificial culture of marine plankton organisms. *J. Marine Biol. Assoc. United Kingdom*, 8, 421-474.
- Bakken, P. 1952. En eksperimentell undersøkelse av temperaturrens innvirkning på fire marine dinoflagellater. Manuscript, University of Oslo, Norway.
- Barker, H. A. 1935. The culture and physiology of the marine dino-flagellates. *Arch. Mikrobiol.*, 6 (2), 157-181.
- Bernard, F. 1939. Recherches sur les Coccolithophorides. I. Principales espèces du plancton à Monaco. *Bull. inst. oceanog.*, No. 767, 1-19.
- Birkenes, E., and T. Braarud. 1952. Phytoplankton in the Oslo fjord during a "*Coccolithus huxleyi*-summer." *Avhandl. Norske Videnskaps-Akad. Oslo I. Mat.-Naturv. Kl.* 1952 (2), 1-23.
- Braarud, T. 1937. A quantitative method for the experimental study of plankton diatoms. *J. conseil, Conseil permanent intern. exploration mer*, 12 (3), 321-332.
- . 1951. Salinity as an ecological factor in marine phytoplankton. *Physiol. Plantarum*, 4, 28-34.
- Braarud, T., and E. Fagerland. 1946. A coccolithophoride in laboratory culture *Syracosphaera carterae* n.sp. *Avhandl. Norske Videnskaps-Akad. I. Mat.-Naturv. Kl.*, 1946 (2), 1-10.
- Braarud, T., K. Ringdal Gaarder, and J. Grøntved. 1953. The phytoplankton of the North Sea and adjacent waters in May 1948. *Rapp. proc. verb.: Conseil permanent intern. exploration mer*, 133, 1-87.
- Braarud, T., and I. Pappas. 1951. Experimental studies on the dino-flagellate *Peridinium triquetrum* (Ehrb.) Lebour. *Avhandl. Norske Videnskaps-Akad. I. Mat.-Naturv. Kl.*, 1951 (2), 1-23.
- Braarud, T., and E. Rossavik. 1951. Observations on the marine dino-flagellate *Prorocentrum micans* Ehrb. in culture. *Avhandl. Norske Videnskaps-Akad. I. Mat.-Naturv. Kl.*, 1951 (1), 1-18.
- Carter, N. 1938. New or interesting algae from brackish water. *Arch. Protistenk.* 90 (1), 1-68.
- Cowley, C. B. 1956. A preliminary investigation of the variation of vitamin B₁₂ in oceanic and coastal waters. *J. Marine Biol. Assoc. United Kingdom*, 35, 609-620.
- Droop, M. R. 1954. A note on the isolation of small marine algae and flagellates for pure cultures. *J. Marine Biol. Assoc. United Kingdom*, 33, 511-514.
- . 1957. Auxotrophy and organic compound in the nutrition of marine phytoplankton. *J. Gen. Microbiol.*, 16 (s), 286-293.
- . 1958a. Requirement for thiamine among some marine and supralittoral Protista. *J. Marine Biol. Assoc. United Kingdom*, 37, 323-329.
- . 1958b. Optimum relative and actual ionic concentrations for growth of some euryphyline algae. *Verhandl. intern. Ver. Limnol.*, 13, 722-730.

- Gran, H. H. 1929. Investigation of the production of plankton outside the Romsdalsfjord, 1926-1927. *Rapp. proc. verb.: Conseil permanent intern. exploration mer*, 56, 1-112.
- Gran, H. H., and T. Braarud. 1935. A quantitative study of the phytoplankton in the Bay of Fundy and the gulf of Maine (including observations on hydrography, chemistry and turbidity). *J. Biol. Board Can.*, 1 (5), 279-467.
- Grøntved, J. 1949. Investigations on the phytoplankton in the Danish Waddensea in July 1941. *Medd. Komm. Havundersøg. København Ser. Plankton*, 5 (2), 1-55.
- Gross, F. 1940. The development of isolated resting spores into auxospores in *Ditylum brightwellii* (West.). *J. Marine Biol. Assoc. United Kingdom*, 24, 375-380.
- Gross, F., and E. Zeuthen. 1948. The buoyancy of plankton diatoms: A problem of cell physiology. *Proc. Roy. Soc. Edinburgh*, B135, 382-389.
- Halldal, P. 1958. Action spectra of phototaxis and related problems in Volvocales, Ulva-gametes, and Dinophyceae. *Physiol. Plantarum*, 11 (1), 118-153.
- Harvey, H. W. 1939. Substances controlling the growth of a diatom. *J. Marine Biol. Assoc. United Kingdom*, 23, 499-520.
- Hasle, G. Rytter. 1950. Phototactic vertical migration in marine dinoflagellates. *Oikos*, 2 (2), 162-176.
- . 1959. A quantitative study of phytoplankton from the equatorial Pacific. *Deep-Sea Research*, 6 (1), 38-59.
- Jenkin, P. M. 1937. Oxygen production by the diatom *Coscinodiscus excentricus* Ehrb. in relation to submarine illumination in the English Channel. *J. Marine Biol. Assoc. United Kingdom*, 22, 301-343.
- Kain, J. M., and G. E. Fogg. 1958. Studies on the growth of marine phytoplankton. I. *Asterionella japonica* Gran. *J. Marine Biol. Assoc. United Kingdom*, 37, 397-413.
- Lanskaja, L. A., and S. J. Sivkov. 1950. On the relationship between the rate of reproduction in diatoms and the quantity of radiation (in Russian), *Doklady Akad. Nauk. S.S.S.R.*, 73 (3), 581-584.
- Lohmann, H. 1908. Untersuchungen zur Feststellung des vollständigen Gehaltes des Meeres an Plankton. *Wiss. Meeresuntersuch.*, N.F. Abt. Kiel, 10, 131-370.
- Marshall, S. M., and A. P. Orr. 1928. The photosynthesis of diatom cultures in the sea. *J. Marine Biol. Assoc. United Kingdom*, 15 (1), 321-364.
- Miquel, P. 1890-93. De la culture artificielle des diatomées. *Diatomiste* I. J. Tempère, Paris.
- Mjaaland, G. 1956. Some laboratory experiments on the coccolithophorid *Coccolithus huxleyi*. *Oikos*, 7 (2), 251-255.

- Nordli, E. 1957. Experimental studies on the ecology of Ceratia. *Oikos*, 8, (2), 201-265.
- Ostenfeld, C. Hansen. 1913. De danske farvandes plankton 1898-1901. Phytoplankton og protozoer. I. Phytoplanktonets livskaar og biologi, samt de i vore farvande iagttagne phytoplanktonarters optraeden og forekomst. *K. Danske Videnskab. Selskab Naturv.-Math.* 9 (2), 113-478.
- Paasche, E. 1959. Fytoplanktonet og den biologiske bakgrunn for primaerproduksjonen i Norskehavet i mai og juni 1954. Manuscript, University of Oslo, Norway.
- Parke, M. 1959. Electron microscope observations on scalebearing Chrysophyceae. *IX Intern. Botan. Congr.*, Sec. 3.
- Provasoli, L. 1958. Growth factors in unicellular marine algae. *Perspectives in Marine Biology*. Symposium 1956 at Scripps Institution of Oceanography. A. A. Buzzati-Traverso, Editor. University of California Press, Berkeley, Calif.
- Provasoli, L., and O. J. Pintner. 1953. Ecological implications of *in vitro* nutritional requirements of algal flagellates. *Ann. N.Y. Acad. Sci.*, 56 (5), 839-851.
- Provasoli, L., J. J. A. McLaughlin, and I. J. Pintner. 1954. Relative and limiting concentrations of major mineral constituents for the growth of algal flagellates. *Trans. N. Y. Acad. Sci.*, Ser. 2, 16, (8), 412-417.
- Provasoli, L., J. J. A. McLaughlin, and M. R. Droop. 1957. The development of artificial media for marine algae. *Arch. Mikrobiol.*, 25, 392-428.
- Ramsfjell, E. 1954. Fytoplanktonet i den nordligste delen av Norskehavet i begynnelsen av juni 1952 og 1953. Manuscript, University of Oslo, Norway.
- Redfield, A. C. 1958. The inadequacy of experiment in marine biology. *Perspectives in marine Biology*, A. A. Buzzati-Traverso, Editor. University of California Press, Berkeley, Calif.
- Rodhe, W. 1948. Environmental requirements of fresh-water plankton. *Symbolae Botan. Upsalienses*, 10 (1).
- Schreiber, E. 1927. Die Reinkultur von marinen Phytoplankton und deren Bedeutung für die Erforschung der Produktionsfähigkeit des Meerwassers. *Wiss. Meeresuntersuch. Abt. Helgoland*, 16 (10), 1-34.
- Spencer, C. P. 1952. On the use of antibiotics for isolating bacteria-free cultures of marine phytoplankton organisms. *J. Marine Biol. Assoc. United Kingdom* 31 (1), 97-106.
- Steemann Nielsen, E. 1952. The use of radioactive carbon (C^{14}) measuring organic production in the sea. *J. conseil, Conseil permanent intern. exploration mer*, 18, 117-140.
- . 1958a. Experimental methods for measuring organic production in the sea. Symposium on measurements of primary production in

- the sea. *Rapp. proc. verb.: Conseil permanent intern. exploration mer*, 144, 38-46.
- Steemann Nielsen, E. 1958b. Light and the organic production in the sea. *Rapp. proc. verb.: Conseil permanent intern. exploration mer*, 144, 141-148.
- Steemann Nielsen, E. and E. A. Jensen. 1957. Primary oceanic production. The autotrophic production of organic matter in the oceans. *Galathea Rept. 1*, 49-136.
- Stosch, H. A. v. 1954. Die Oogamie von *Biddulphia mobiliensis* und die bisher bekannten Auxosporenbildungen bei den Centrales. *Congr. intern. botan., 8^e Congr. Paris*, 17, 58-68.
- Sweeney, B. M. 1954. *Gymnodinium splendens*, a marine dinoflagellate requiring vitamin B₁₂. *Am. J. Botany*, 41 (10), 821-824.
- Välikangas, I. 1926. Planktologische Untersuchungen im Hafengebiet von Helsingfors. I. Über das Plankton insbesondere das Netz-Zooplankton des Sommerhalbjahres. *Acta Soc. Fauna Flora fenn.*, 1