Reprint

OIKOS

ACTA OECOLOGICA SCANDINAVICA

Vol. 8 Fasc. 2 1957

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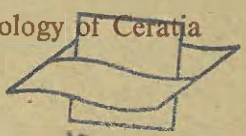
Experimental Studies on the Ecology of Ceratia



REDIGENDA CURAVIT:

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EJNAR MUNKSGAARD — COPENHAGEN

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is an ecological journal issued by ecologists in Denmark, Finland, Iceland, Norway and Sweden.

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The subscription of OIKOS is 40 Danish Crowns per annum.

The journal is distributed solely by Ejnar Munksgaard, Publishers, Copenhagen, Denmark, not by the editors. Orders may be placed with any bookseller all over the world or direct with the publishers.

The chief editor is Dr. C. Overgaard Nielsen, Molslaboratoriet, Femmøller, Jylland, Denmark.

OIKOS 8:II 1957

EXPERIMENTAL STUDIES ON THE ECOLOGY OF CERATIA

BY

ERLING NORDLI

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Preface

This work contains the results of investigation and experimentation carried out from 1950 to 1956, during which time I held a grant from the Norwegian Research Council for Science and the Humanities. Supplementary data from investigations carried out for my cand. real. thesis are also presented.

Collection of material took place, for the most part, at the Biological Station in Drøbak where Professor Bjørn Føyn, placed working space and equipment at my disposal for several summers and for a series of shorter visits. Expenses incurred at the Station were in part covered by a stipend from The Hielmstierne Rosencroneske legacy.

The experimental part of the work was carried out mainly at the Institute for Marine Biology, Section B, University of Oslo, where Professor Trygve Braarud, made available working space and equipment. Through the whole investigation he offered advice on setting up the experiments and gave me a great deal of assistance in discussion of the results and writing of the manuscript.

The figures were drawn by Mrs. Bjørg Paulsen. Mrs. Harriett Holm Hansen and Mrs. Norma Smayda kindly helped in translating the manuscript. The Norwegian Research Council for Science and the Humanities covered part of the printing costs.

I wish to express my sincerest thanks to the above-mentioned persons and institutions.

I. Previous Investigations on the Ecology of Ceratia

Since the ceratia are of a very wide distribution, material to determine their exact occurrence had to be collected from restricted areas as well as from extensive expeditions covering one or more oceans. The most important of these

were the "Meteor" 1925–27, the "Carnegie" 1928–29, and the "Dana" 1928–30 expeditions.

This material confirmed Schütt's observation in 1893 that the greatest number of *Ceratium* species was found in tropical oceanic areas. It also showed that compared with other dinoflagellates and the diatoms, the number of *Ceratium* cells per unit area is generally rather low.

In an attempt to obtain an indication of which factors delimite this distribution of the different *Ceratium* species, the ecology of ceratia has been discussed on the basis of available hydrographical and biological data. The influence of the more important factors are reviewed in the following sections.

The salinity factor

The slight range in oceanic water salinity which is such a good indication of different water masses seems to influence the distribution of ceratia to a negligible degree only. PETERS (1934) could demonstrate no influence from salinity differences in the Southern Atlantic; neither could STEEMANN NIELSEN (1934) in the Southern Pacific. SCHUBERT (1937) reports that 11 *Ceratium* species mentioned by name, are found only at salinities exceeding 35.8 $^0/_{00}$. STEEMANN NIELSEN (1939), on the other hand, observed that many of these species are found at least down to a salinity of 33.5 $^0/_{00}$, and a few go below 33.0 $^0/_{00}$. Later investigations have confirmed this: "In the Carnegie investigations no correlation could be found between the salinity and the distribution of any species. Thus, apparently these species are not affected by such slight variations in salt content as are found in the open ocean." (GRAHAM and BRONIKOVSKY 1944, p. 7). Whether or not this statement is valid for all species, even where fluctuation in salinity is greater (e. g., in neritic areas) has not been ascertained. However, *Ceratium tripos*, *C. fusus* and *C. furca* are found in the North Sea, the Transition Area and the Western Baltic, within very wide ranges of salinity, ordinarily down to 9–10$^0/_{00}$, occasionally even lower (APSTEIN 1910, STEEMANN NIELSEN 1940, AURIVILLIUS 1896, OSTENFELD 1913).

The temperature factor

The influence of temperature on the distribution of *Ceratium* species has been discussed by several investigators, all of whom have come to about the same conclusion. CLEVE (1899) lists ceratia among the predominant forms in his plankton types and reports at which temperatures the different species are found. GRAN (1902) subdivides the peridineans into Arctic, Boreal and temperate Atlantic species among which ceratia are leading forms. OSTENFELD (1913) found in his material that ceratia present in an area could survive when the

temperature dropped below that required for their occurrence at the beginning of the season. Even though they draw different borders, PETERS (1934) and STEEMANN NIELSEN (1934; 1939) agree that temperature is of importance for the distribution of *Ceratium* species. GRAHAM and BRONIKOVSKY (1944) subdivide ceratia into groups according to temperature tolerance: Intolerant Tropical (> 19° C), Slightly Tolerant Tropical, Very Tolerant Tropical, Cosmopolitan, and Subpolar.

Some of these data for minimum temperatures and temperature ranges from natural populations are given in Table 1. The four species selected are those dealt with in the experimental part of this paper.

Table 1.

Species	Ostenfeld (1913)	Cleve (1900)	Graham & Bronikovsky (1944)	
	Min. °C	Min. °C	Min. °C	Max. °C
Ceratium tripos	– 1.2	2.0	11.4	29.4
– *fusus*.....	– 0.8	3.0	2.1	29.5
– *furca*	– 1.2	2.0	6.6	29.4
– *lineatum*.....	—	3.15	6.7	21.2

The effect of nutrient salts

Differing opinions have been advanced concerning the influence of nutrient salts, especially phosphate, upon the distribution of ceratia. PETERS (1934) and GRAHAM and BRONIKOVSKY (1944) maintain they have found a certain correlation between low nutrient salt content and high number of species, but since the dependence is doubtful Graham and Bronikovsky take extreme reservations. STEEMANN NIELSEN (1934) could prove no connection between phosphate content and number of species.

Quantitative phytoplankton investigations in the North Sea and the Baltic have demonstrated that the common *Ceratium* species have their maxima in the period August-December. (CLEVE 1900, GOUGH 1905, LOHMANN 1908, APSTEIN 1910, BRANDT 1921). All-year examinations of the sea water in the areas mentioned have ascertained that the phosphate content increases during the period when the ceratia form a substantial part of the phytoplankton (SCHREIBER 1927, GESSNER 1933, WATTENBERG and MEYER 1936). Since the number of species varies very little and seems to be wholly independent of the phosphate concentration, in these areas there seems to be no reason to assume that high concentration of phosphate inhibits the growth of *Ceratium*.

The fact that the diatom plankton is often succeeded by a *Ceratium* plankton might depend upon their different temperature optima and the ceratia's ability

to survive at a lower concentration of nutrient salts, although the latter point is not certain. The vertical migration that the ceratia are able to undertake (lately proved to be phototactic, HASLE 1950) also might aid them in securing nutrient salts from more extensive water layers. Unfortunately, simultaneous determinations of the nutrient salt content and quantitative measurements of the *Ceratium* population are completely lacking for most species, so there is insufficient material at hand to solve the problem.

The influence of plankton metabolites

As mentioned above, the greatest variety of *Ceratium* species is found in the open ocean and decreases considerably in neritic areas. STEEMANN NIELSEN (1934) assumes that this fact depends upon plankton metabolic products which are found in higher concentrations in the shallow waters along the coasts than over the depths of the oceans, and that only a few *Ceratium* species can endure these higher concentrations. On this problem GRAHAM and BRONIKOVSKY write: "Since no measurement of such substances are available, it is impossible to test this theory." (1944, p. 10).

The light factor

Since the ceratia are phototactic they probably situate themselves in depths of optimal light intensity and/or quality, eventually finding the best conditions obtainable in an area. As HASLE (1950) points out, this light optimum probably varies for the different species. STEEMANN NIELSEN (1934) distinguishes a number of *Ceratium* species favouring shade. At the same time he reviews and discusses the rather exiguous and in part contradictory information then available on the depth distribution of ceratia.

Transport by ocean currents

Passive transport of individuals by currents influences the distribution of *Ceratium* species by exposing the plankton to changes in temperature and salinity brought about by the mixing of different water masses. Under such alterations in their environment the ceratia survive as long as conditions are suitable. Owing to extensive current systems, therefore, few geographical races of ceratia can be expected, but the very tolerant species should be widely distributed. Their only restriction, as pointed out by STEEMANN NIELSEN (1939), is the high temperature of the tropical oceans which acts as a barrier between the cold water forms of the two hemispheres.

Previous attempts at cultivation and the conditions employed

Certain problems occurring in connection with *Ceratium* ecology can be treated experimentally. In earlier literature some of these have been pointed out. GRAN (1902, p. 45), using statistical methods, investigated whether morphologically related specimens are systematically different or only temperature variants of the same species, "Da es bis jetzt nicht möglich gewesen ist, diese Formen auf längerer Zeit in Kultur zu halten ...". JØRGENSEN (1911) discussed the size variation of some *Ceratium* species under different temperatures and salinities, and assumed that this variation might depend upon varying division rates influenced by varying ecological conditions. He further suggested that this hypothesis could be tested experimentally. In the discussion of whether the variability of *Ceratium tripos* is genotypical or contingent upon ecological factors, STEEMANN NIELSEN (1939, p. 18) writes: "Wenn man dazu kommen sollte, die Ceratien kultivieren zu können, wird man diese Frage lösen können."

A few attempts have been undertaken to keep ceratia in cultures, but as a rule they have proved unsuccessful. In the earliest investigations unialgal cultures were not employed.

The first *Ceratium* culture attempts reported were those of LOHMANN (1908). He kept mixed plankton in big glass jars and found in these cultures chains of *Ceratium tripos* which previously had not been observed in the area from which he collected his samples. By keeping sea water with its natural plankton population in flasks floating on the sea surface, GRAN (1908) made experiments with mixed plankton, of which ceratia formed a considerable part. His experiments were undertaken both for estimation of division rates and for control of the influence of nutrient salts. TSCHIRN (1920) mentioned in his dissertation (available only as an abstract) that he tried to cultivate ceratia, but had little success. The reason for the negative result, he supposed, was failure in finding the proper light quality for these "Schattenpflanzen des Meeres".

PETERS (1929) kept mixed cultures of dinoflagellates, including ceratia, for a study of locomotory and flagellar movement. By keeping crude plankton standing for sedimentation he got rid of the bulk of diatoms. The Crustacean larvae and Copepoda gathered on the illuminated side of the vessel where they were easily omitted when the dinoflagellates together with the ciliates were pipetted off. In these mixed cultures Peters observed stages of division and chain formation in the ceratia which had taken place during the experiment.

BARKER (1935, p. 164) was the first to keep species-pure cultures of *Ceratium* for a long period. (His *Ceratium 2* is closely related to, if not identical with *Ceratium tripos* Müller). Some of the cultures were successful, but not infrequently the cells became "much distorted and knarled". "Considerable experi-

mental work has been done with this organism", he writes (p. 165) although no publications treating this topic seem to have appeared. GROSS (1937) tried to keep cultures of *Ceratium tripos*, *C. fusus* and *C. furca*. A few days after isolation the flagellates were more active than in the original plankton sample, but in the course of time the cultures died out.

Various media for *Ceratium* cultures have been tried: Natural sea water with added nutrient salts (GRAN 1908) and without (LOHMANN 1908, PETERS 1929), Miquel sea water (BARKER 1935) and "Erdschreiber" (GROSS 1937).

TSCHIRN (1920) assumed that the negative results of his attempts at cultivating ceratia were due to incorrect illumination. However, BARKER (1935) in all his cultures (not specifically *Ceratium*) employed artificial illumination as well as daylight with equally good results.

GRAN (1908) used temperatures which were identical with those of the sea water from which he took his plankton samples. In cases where other experimenters have recorded temperature conditions, room temperature was used for laboratory cultures.

Success in cultivating ceratia seems to depend upon the handling of the cells. BARKER (1935) obtained successful cultures by using relatively large amounts of water and few washings of the cells when starting cultures. GROSS (1937) washed the cells many times when starting cultures and once or twice before transferring the cells to fresh culture media; the organisms were transferred daily, but within a week all the cells died.

II. Material and Methods

Organisms employed

The *Ceratium* species used in the experiments described below are:

1. *Ceratium tripos* var. *atlanticum* (Ehrenberg) Ostenfeld.
2. *C. fusus* var. *seta* (Ehrenberg) Jørgensen.
3. *C. furca* (Ehrenberg) Claparède & Lachmann.
4. *C. lineatum* (Ehrenberg) Cleve.

All species were isolated from sea water samples collected in Drøbak Sound, Oslofjord.

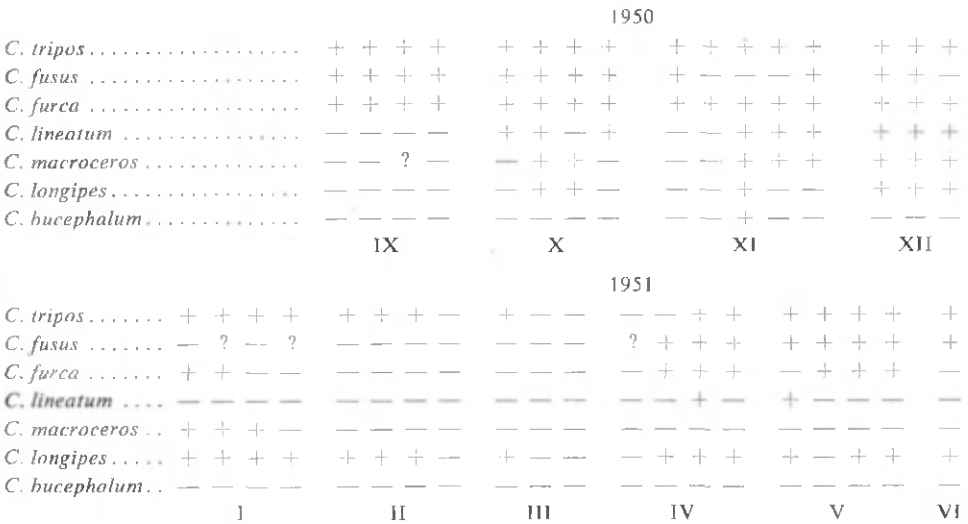
Since the original cultures of *C. tripos*, *C. fusus* and *C. furca* died out, probably because of unchecked high temperatures in the laboratory during a heat period, new clones for these species were necessary. The isolation dates and the life periods of the cultures are listed below. The second *C. furca* culture was available immediately, as it had been started in July 1950 and kept at the Biological Station in Drøbak where the temperature in May 1951 was not lethal.

First *C. tripos* culture, July 1948 – May 1951.
Second – – May 5, 1953 – August 1955.
First *C. fusus* – July 15, 1947 – May, 1951.
Second – – August 15, 1952 – August 1955.
First *C. furca* – July 1949 – May 1951.
Second – – July 1950 – July 1956.
C. lineatum – January 3, 1952 – August 1955.

(Two of the four species, *C. furca* and *C. lineatum*, are comparatively easy to keep in culture since they behave more or less like the other marine dinoflagellates which have been kept in culture. These two species, therefore, might be added to the list of marine dinoflagellates suitable for experimental work. The clone of *C. lineatum* has been kept in culture for several years and has proved to be a true *C. lineatum* and not the "lineata" form of *C. tripos*. The latter has been isolated repeatedly from *C. tripos* culture but thus far with no success in giving subcultures.)

All cultures were isolated from sea water samples collected in the Drøbak Sound of the Oslofjord. In this locality *Ceratium tripos* is an all-year species, *C. fusus* and *C. furca* are usually absent in winter, while *C. lineatum* is seldom

Table 2.



found during the summer. Table 2 shows the occurrence of ceratia in Drøbak Sound based on a nearly complete series of weekly net tows during the period from September 1950 to June 1951. Since the phytoplankton population alters

from one year to another the data in the figure are not exhaustive. The hauls were originally carried out to determine the occurrence of *Halosphaera viridis* in this locality, so the information in respect to ceratia is merely qualitative.

Culture media

Miquel sea water (ALLEN and NELSON 1910) and "Erdschreiber" (FØYN 1934) were used as media for stock cultures. Both of these have proved efficient, but because the precipitate in Miquel sea water is troublesome in *Ceratium* cultures, "Erdschreiber" was largely employed. Where the character of the experiment required it, the "Erdschreiber" was modified and recorded in the descriptions of the experiments. When not otherwise specified, "aged sea-water" from Drøbak Sound was used in preparation of the culture media.

Light conditions

For illumination diffuse daylight, ordinary electric bulbs and Philips' fluorescent tubes were employed. All of these light sources are suitable to the cultures, but, probably due to the special relation between light conditions and the division period of ceratia, daylight seemed to be best (BERGH 1886, GOUGH 1905, APSTEIN 1910, HASLE and NORDLI 1951). Since daylight is not satisfactory for quantitative experiments owing to the fluctuation of its intensity, artificial illumination was generally used. The laboratory equipment did not permit the use of day and night periods of light in the experiments. The conditions are recorded for each experiment. Where light intensities were measured, a photocell was employed.

Culture vessels

Jena and Pyrex glass flasks were used, various available types and sizes for stock cultures, and 50 ml extraction flasks for experiments, when not otherwise stated. All glass-ware employed was washed in a standard way.

Methods for starting and maintaining cultures

As stated, previous literature on the cultivation of ceratia has been extremely scant. Until now no safe culture technique has been presented, but many of the culture attempts reported have yielded information about different techniques. The methods described below were not fully developed when the viable cultures of this investigation were first obtained. Thus the description includes experiences gained during the period of culture as well as information obtained during the numerous experiments.

For the following reasons the classical methods for obtaining clone cultures of plankton algae by repeated washings of a single cell or by dilution have not proved suitable for ceratia.

1. When outside conditions are not carefully controlled capillarial drop to drop transfer to a slide, owing to the limited heat capacity of such a small volume of water, might cause rapid temperature fluctuations in the solution. Also the subsequent evaporation would cause a rapid change in the salinity. Furthermore, the narrow capillaries which must be used when working with small amounts of water appear to be injurious to the relatively large *Ceratium* cells, especially when the transfers are repeated several times.

2. Because numerically the ceratia form such a small fraction of the organisms contained in a plankton sample, and because the percentage of cells surviving transfer from sea water to the culture medium is low, the number of cultures necessary with the dilution method would exceed a practicable quantity in regard to both place and control. Also, due to the low division rate of ceratia, this method would require an impractically long period before any successful cultures could be recognized.

To obtain successful isolations of ceratia, 1) the cells must be given extremely careful mechanical handling, 2) only a minimum change in temperature and salinity should be allowed, 3) control methods should be set up so that the success of the cultures can be determined within a short period. In this investigation the fulfillment of these requirements was attempted in different ways: through the use of culture methods described in previous literature, through eventual alterations of these methods, and through the development of new methods as the work progressed.

Since ceratia are relatively large organisms they can be isolated at low magnification under a dissecting microscope, making it possible to examine a water volume of several millilitres within a short period. In this investigation a Pasteur pipette was modified and used for isolating (cfr. GROSS 1937, p. 755). The stock of the glass tube was cut and the two parts reconnected at some distance by means of a piece of rubber tubing; the opening formerly covered by an elastic bulb was sealed. The length of the capillary section was reduced to 1½–2 cm. In this way the pipette could be manipulated like a pencil, the pressure of two fingers regulating suction while the whole hand controlled its movement.

Instead of the watch-glasses used by Gross, Petri dishes with 5–10 times the amount of water were employed. This reduced the number of transfers necessary and, to a certain degree, checked the temperature fluctuation. The relatively large amount of water also lessened the alteration of salinity through evaporation.

To provide a nutrient solution of proper salinity, sea water was collected where the plankton samples were taken for the isolations. The nutrient solution was cooled to the sea temperature. In order to ensure a minimum difference

between laboratory temperature conditions and those of the sea, the isolations were made at high sea temperatures whenever possible. However if an organism was found only at a low temperature, laboratory conditions were altered accordingly.

As the smallest possible number of transfers is preferable, isolations are most easily done when the ceratia are abundant relative to other organisms. Such conditions are found especially during the summer. Then the ceratia can be isolated directly from unconcentrated sea water samples, thus avoiding influences from filtering through a net.

To prevent the number of culture flasks from being impractically high, the primary isolation, which has proved to be the most critical because of repeated washings, was done by collecting many cells, preferably 50–100, in each of several flasks. Though only a small number of cells survived in these multicellular cultures, unicellular cultures could be isolated from them with a single transfer, and, accordingly, with small risk of injuring the cells. (In all such re-isolations from different mass cultures, positively different clones are obtained.)

The growth of the cultures was controlled in the culture vessels themselves. 50 ml extraction flasks were employed, since their contents could be examined under a binocular dissection microscope having a sufficient working distance and wide field of vision. (Later models by Leitz, Spencer and Zeiss fulfill these demands.) In this way unsuccessful isolations could be rejected after a few days, and positive ones kept under control without disturbing the cells. Because of the low division rate of ceratia, the ordinary method of examining a small amount of the fluid would have given a dependable positive or negative result only after a much longer period.

Contaminating organisms with higher division rates could still be controlled through examination of small samples in an ordinary microscope, and through indications given by a discolouring of the culture fluid.

Control methods for experiments and calculation of results

Various methods were employed to determine growth in the experiments:

1. Counting of the living cells in the culture vessels during the experiment. By this method a change in division rate during the growth period is revealed. The reliability of the method depends upon the speed at which the organisms move, the amount of culture fluid and the magnification required.

2. Total counts of preserved cultures by means of an inverted microscope (UTERMÖHL 1931). Theoretically this method is free from statistical errors and is employed when practicable.

3. Counting of greater or lesser fractions of the preserved culture, depending

on culture density. The reliability of this method can not be controlled in each case, but in this investigation the number of cells or the volume of the culture fluid were of sufficient magnitude to keep the error under a safe level.

Details of the methods are given for individual experiments. Where the character of the experiments was suitable, the reliability of the results was tested by a variance analysis according to MATHER (1951), and only creditable results accepted. This analysis gave no information as to why an experiment was unsatisfactory, but it was assumed that as a rule faulty culture techniques were the cause.

Most of the experiments were carried through with several parallels, usually five, and the average number of cells was used to calculate the division rate. Under the assumption that optimal conditions cause maximal division rate, in experiments which included one or more treatments these division rates were used as a criterion of effect. Since the experiment periods were of varying length, the calculations were made for divisions per 24 hours and were carried to two decimal places.

III. Description of experiments and investigations

The *Ceratium* cultures employed in the experiments described below were species-pure cultures, but not bacteria-free. Such cultures could be used in the quantitative experiments since only those factors were treated which were comparable to natural phenomena where the influence of bacteria is regarded as a part of the ecological system.

A. Experimental studies of the culture technique

1. Differences between unicellular and multicellular cultures.

Experiments with different species and under various conditions were carried out to discover whether growth conditions in cultures are comparable to natural conditions. Cell mortality from transfers was investigated in unicellular as well as multicellular cultures. Some of the experiments were arranged in such a way as to give control of division rates; this will be described later.

Ceratium tripos. 10 cells were inoculated into each of six flasks containing 30 ml "Erdschreiber". The flasks were placed in a north window, light intensity not measured. During the experiment the organisms were counted in the culture flasks, so the figures for the first few days when the number of cells was low are probably more dependable than those for the later days (Table I). On the 17th day the cultures were preserved and the exact cell numbers determined.

A growth experiment with multicellular cultures in two series of five flasks, each containing 30 ml "Erdschreiber", was performed under artificial illumina-

tion ("Electric sun", Pringsheim's model). The cultures were not controlled during the experiment period, but were counted totally afterwards (Table II).

The experiment for Table III was carried out by daylight in a north window, light intensity not measured. In each of five flasks containing 30 ml "Erdschreiber" one single cell was inoculated. The organisms were counted in the flasks during the experiment period, and accurate counts were made at its termination.

C. fusus. A preliminary experiment with low and high initial numbers of cells was carried through (Table IV). The series with an initial number of five cells was counted by inoculation. In the second series the initial number of 235 represents the average cell content in one ml of the stock culture; this was found by counting 5 one ml samples which contained 243, 248, 230, 234 and 220 cells respectively. One culture failed for some reason and is omitted in the final calculation. After all the cultures were preserved, five ml samples from each of them were counted. The cultures with an initial number of five cells were counted in toto.

A series of experiments under artificial illumination was made with initial cells numbering one, ten and fifty in each of five parallels of "Erdschreiber". All the cultures were preserved after the termination of the experiment and the cell numbers found by counting the total volume. Two of the unicellular cultures did not grow and are omitted in the calculation of the division rates (Table V).

C. furca. In the experiments with multicellular cultures were employed cells isolated directly from sea water and transferred to "Erdschreiber" made from non-stored sea water. The 14-day culture was counted in toto, but the 21-day and 27-day cultures were counted by one ml samples (Table VI).

Unicellular cultures of *C. furca* were controlled during the growth period by counting the cells in the living cultures, but their moving habit and the degree of magnification required made the intermediate results uncertain. The end result, however, was accurately determined by a total count. The nutrient solution and the preparation of the cultures was as above (Table VII).

Owing to the difficulties which arose in controlling the growth in *C. furca* cultures in 50 ml extraction flasks during the experiment period, an assay was made with smaller vessels and less fluid. 40 mm square glass staining vessels containing two ml of culture fluid each were used as culture vessels. Closely fitted glass covers prevented evaporation. Growth took place in four of the six parallels (Table VIII).

C. longipes. This organism was not employed in other experiments, since it has hitherto refused all efforts of keeping persistent stock cultures. The experiment was done with cells isolated directly from sea water. It is presented here because it renders material for comparison with data known from natural populations, and for comparison with other cultures (Table IX).

2. The rate of growth in relation to growth period and culture density.

For *Ceratium tripos*, *C. furca* and *C. lineatum*, experiments were made to ascertain whether or not growth rate depends upon growth period and culture density. For all organisms stock cultures diluted with "Erdschreiber" were used for experimental cultures, and the cell content per ml was counted. The diluted stock culture was divided into 30 ml quantities and placed in each of 16 Erlenmeyer flasks, four each of 50, 100, 250 and 500 ml capacities. To the 100, 250 and 500 ml flasks was added 30, 90 and 210 ml "Erdschreiber" respectively, thus forming a dilution series with cell content per ml in the proportions 1/1: 1/2: 1/4: 1/8. These cultures were grown at room temperature under 1500 Lux illumination. Owing to restricted space under even illumination more parallels could not be used. The results given by the countings, therefore, are not too dependable. The differences between successive counts, however, were large enough to justify using the method. The culture volumes used in determining cell numbers are specified in Tables X, XI and XII. When cultures were too dense to count, dilutions of 1:5 or 1:10 were made, of which at least one ml was counted through.

C. tripos. One series of countings every seven days did not cover the exponential growth phase in either of the flasks so another one with countings every 14 days was started. The initial number per ml in the 30 ml cultures was 94 cells. Cell numbers and division rates in the cultures after 14, 28, 42 and 56 days are given in Table X.

C. furca. Cultures were preserved and counts made every seven days. Cell numbers and division rates are given in Table XI.

C. lineatum. The same culture period as for *C. furca*. Cell numbers and division rates are given in Table XII.

Similar experiments with *C. fusus* did not give any definite results since cell mortality within the cultures completely obscured the effect of treatments.

Discussion. The comparison of growth rates in unicellular and multicellular cultures showed that under similar conditions the former have higher division rates than the latter. The majority of the experiments, however, were performed under light conditions which could not easily be controlled; therefore the comparison between different experiments has low significance. The high number of unsuccessful unicellular cultures, some from experiments which were rejected as a whole, shows that a certain mortality from inoculation might cause multicellular cultures to give a lower division rate; in other words the actual initial number is different, and lower, than that used in the calculations.

Comparison with division rates found in material from natural surroundings shows that culture conditions were not ideal, but were good enough to be used

in the investigations, especially since the relative values of the effect of various treatments were of primary interest.

A comparison of the division rates in tabular form is given below.

Table 3.

Species	Cultures		Maximal values found by		
	Multicellular	Unicellular	Gough	Gran	Apstein
C. tripos	0.11–0.32	0.24–0.33		0.37	0.27*)
C. fusus	0.11–0.18	0.40	0.62	0.47	0.18
C. furca	0.36–0.47	0.37–0.54		0.46	0.32
C. longipes		0.14–0.29			0.36

*) Not maximal value. See text.

The values for comparison were taken from GOUGH (1905), GRAN (1908) and APSTEIN (1910) and recalculated from division percentage to divisions per 24 hours. Apstein's maximal value for *C. tripos* was not used since, according to him, it was influenced by unspecified circumstances. Instead, the average from his most favourable sample was substituted. It is noteworthy that this value lies within the ranges of the table above, for unicellular as well as multicellular cultures. The distribution of values for the cultures was approximately the same as that for natural populations.

Three experiments (Tables I, III and VIII) were organized to compare the division rates with the length of the growth period. Owing to the low division rates, the initial cell numbers, rather than the nearest previous counts, were always used as the basis for calculation. The results are given graphically in Fig. 1. The slightly decreasing division rate indicated by these experiments proved to be so steady that periods of similar duration should give sufficiently reliable results in future experiments.

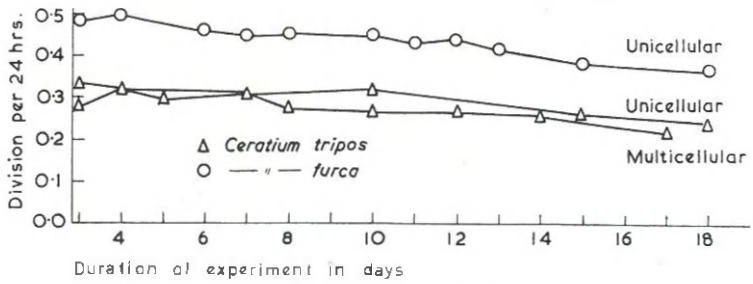


Fig. 1. Division rates of *Ceratium tripos* and *C. furca* in relation to growth period.

It is generally recognized that the growth rate in cultures of unicellular plants can be expressed graphically through a sigmoidal curve starting with a lag phase and continuing with an exponential phase which, after a period of varying length, takes the form of the asymptote and, in an aging culture, eventually decreases.

Experiments with different volumes of inoculum were effected by placing an equal number of cells in different amounts of culture fluid (see p. 213). These were undertaken in order to determine at which cell density per ml the exponential growth phase ends, and whether cell numbers from quantitative experiments lie below this critical point on the growth curve.

The average division rates in cultures of *Ceratium tripos*, *C. furca* and *C. lineatum* are given graphically in Figs. 2, 3 and 4, values taken from Tables X, XI and XII. As growth rates here are expressed in divisions per 24 hours, the exponential phase is represented by points lying at the same level. The influence of a lag phase in the beginning of the growth period, and the decrease of the growth rate in old cultures would lower the average values. The connecting lines should not be understood as representing a growth curve, since each point represents the value from a single culture with a growth period from the beginning of the experiment up to the day given; they were added to facilitate an overall impression.

A lag period could be traced in some of the cultures, and was most clearly expressed in *C. furca*. No regularity for the dependence of the growth rate upon the original inoculum could be found. In *C. tripos* the greatest amount of inoculum gave the highest division rate; in *C. lineatum* the amount of initial cells caused little difference, whereas in *C. furca* the smallest amount of inoculum gave the highest division rate. Nevertheless the influence of the inoculum itself may not be responsible for the different results. There is a possibility that the varying amounts of old culture fluid transferred to the subcultures contained growth promoting or inhibiting substances which acted in different ways upon the three organisms. Also, the prehistory of each of the stock cultures could have made the culture fluids markedly different. Little is known concerning the nature of such influences, and their consequences to growth rates are difficult to estimate. For details about this subject, see LUCAS, 1947.

One source of error was the mortality within the cultures. Examination after 7 days (14 for *C. tripos*) found a number of thecae with no cell contents; many seemed to be more or less disintegrated, so the actual number could not be ascertained. Part of this was probably due to the certain percentage of cells which always die from transfer to subcultures (see p. 210). Also, cells from a diluted stock culture are of all degrees of viability, and consequently are subject to a higher mortality, whereas single cells for inoculation are actively swimming organisms in good condition.

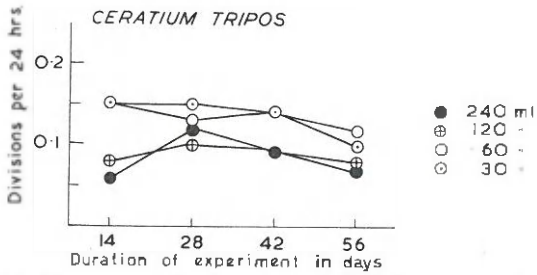


Fig. 2. Division rate of *Ceratium tripos* in relation to growth period and volume of medium.

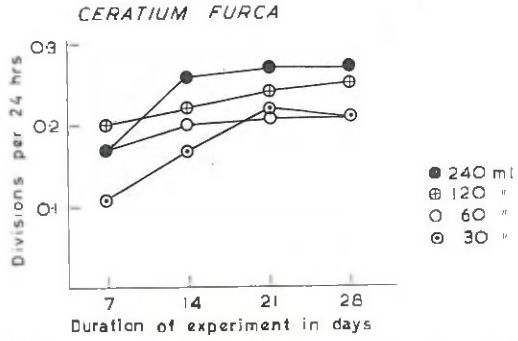


Fig. 3. Division rate of *Ceratium furca* in relation to growth period and volume of medium.

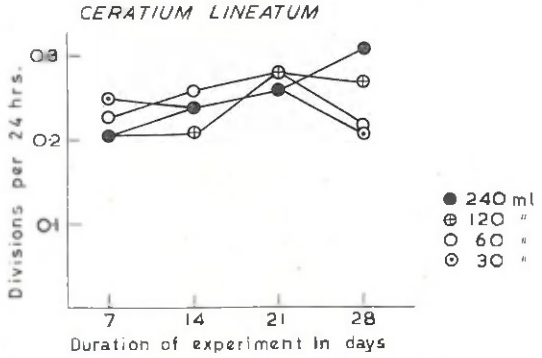


Fig. 4. Division rate of *Ceratium lineatum* in relation to growth period and volume of medium.

Since all the calculations were made on the basis of the inoculum, the earlier countings were probably affected more. The lag phases indicated in some of the cultures, therefore, were probably not as long and pronounced as the figures

suggest. (This is in good accordance with Fig. 1 where no lag period can be traced after the fourth day.)

According to these experiments the culture densities lying within the exponential phase that can be used safely for experimental cultures are 500 cells per ml for *C. tripos*, 1000 cells per ml for *C. furca*, and 5000 cells per ml for *C. lineatum*, providing that the inoculum is of a moderate amount. For *C. furca*, even after 28 days, it could not be determined with certainty that the end of the exponential phase had been reached in the 30 ml cultures. (The above numbers are not certain since their statistical background is unreliable owing to lack of parallels (see p. 213). Cell numbers in quantitative experimental cultures seldom reach 1/10 of these values.)

B. The effect of environmental factors upon the rate of growth

1. Salinity.

Salinity is one of the more important factors in marine ecology. In oceanic areas it is of only indirect biological importance through its influence upon currents and stratification. In coastal waters and fjords its range of fluctuation can be so wide as to be a direct factor in the life of plankton organisms. Attempts to characterize certain *Ceratium* species as stenohaline halophiles have been uncertain and in part contradictory (see p. 202), but some *Ceratium* species have been recognized as euryhaline and can be found in neritic as well as oceanic waters. In this latter group can be placed all ceratia dealt with in this paper.

Since the salinity range in sea water is not wide, growth experiments within a wide range of salinity were constructed to discover whether or not ceratia have a more extended range of tolerance than that covered by natural surroundings, and whether an optimum, if found, lies within salinities of restricted geographical areas.

The salinities used in the experiments extended from $5^0/_{00}$ up to $45^0/_{00}$ in steps of $5^0/_{00}$. To provide a uniform constitution of the nutrient solution, sea water was concentrated through evaporation at 70° C to a salinity between 45 and $50^0/_{00}$. This brine was adjusted to $45^0/_{00}$ through addition of glass-distilled water. From this "$45^0/_{00}$ sea water" Schreiber's solution and "Erdschreiber" were prepared. From glass-distilled water a solution with phosphate and nitrate in proportions as in Schreiber's solution was prepared, respectively with addition of soil extract. Afterwards these solutions could be mixed in any proportion, with differences arising only from the concentration of the elements originating from the sea water, excepting phosphate and nitrate since the concentrations used in Schreiber's solution presumably were high enough to min-

imize the importance of these elements in the sea water used. The salinity was controlled with a salinometer and titrations to an accuracy of $\pm$ 1‰.

Before the experimental cultures were started, the organisms were adapted to the salinities at which they were to be tested, through successive transfers to the different salinity levels where they were kept in cultures until two or more divisions had taken place. When necessary, intermediate salinity levels were used. Five parallels were kept at each salinity level, and either daylight or artificial light employed; specifications are given in the tables. The initial number of cells in each flask was transferred with a pipette from the adaption cultures. The volumes of culture fluid examined are also given in the tables.

Ceratium tripos. The result of the experiment is given in Table XIII. The variance analysis gave a probability of < 0.001 for treatment and 0.25–0.10 for parallels.

C. fusus. Results given in Table XIV. Probability < 0.001 for treatment and > 0.25 for parallels.

C. furca. Results given in Table XV. Probability < 0.001 for treatment and > 0.25 for parallels.

C. lineatum. Results given in Table XVI. Probability < 0.001 for treatment and $0.25 - 0.10$ for parallels.

Discussion. According to variance analyses salinity has an influence upon growth rate.

The results from the four salinity experiments are given graphically in Fig. 5. All the organisms showed maximal division rate at lower salinities than found in oceanic water, and all of them had to be characterized as euryhaline, even though their ranges of tolerance differ. Their optimal salinity which lies around 20‰ is never found in oceanic waters, but is frequently present in fjords and neritic areas. This is in good agreement with observations from natural environments, ceratia seldom being found in considerable numbers in the open ocean. On the other hand, the four species used in the experiments, as well as a few others, are often abundant in northern European coastal waters of low salinity. (Apstein 1910, Cleve 1900, Gran 1915, Steemann Nielsen 1940, Ostenfeld 1913). In the Black Sea, another large area of surface water of low salinity, Jørgensen (1920) found large numbers of *C. fusus* in the upper layers and regarded the occurrence of *C. tripos* south of the Dardanelles as a sharp indicator of water from the Black Sea.

From their relatively high division rates even at 40‰ it is evident that high salinities never will exclude *C. tripos*, *C. fusus* and *C. furca* from any locality in the sea owing to salinity concentration. In fact, *C. fusus* and *C. furca* are found in the Gulf of Oman at a salinity of 39.14‰ (Böhm 1931 a), and, in the eastern Mediterranean, *C. tripos* occurs at 38.64‰ and *C. furca* at 39.22‰ (Jørgensen 1920).

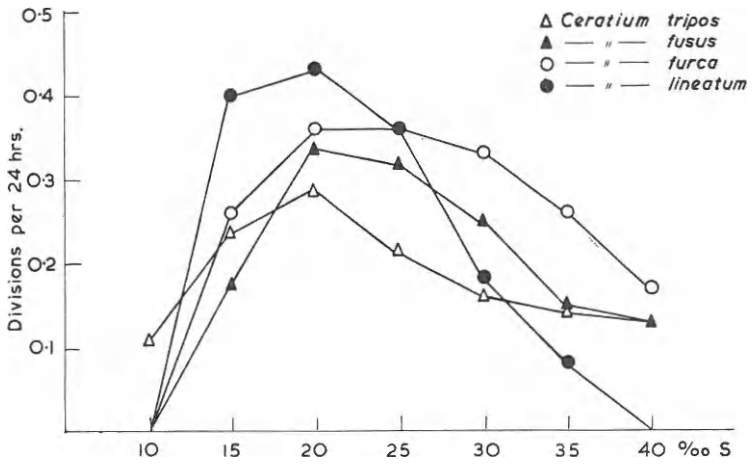


Fig. 5. Division rates at various salinities.

2. Temperature

All *Ceratium* species dealt with in this investigation are found over large areas throughout the year at temperatures ranging approximately from — 1° C to 29.5° C for *Ceratium tripos*, *C. fusus* and *C. furca*, and approximately 3° C to 21° C for *C. lineatum* (see p. 203). They all must be characterized as excessively eurytherme, but their numbers vary greatly with place and season. According to observations from natural surroundings, it is reasonable to assume that the division rates of these organisms are low, sometime negligible, at low temperatures. The number of ceratia is usually low at times when temperature conditions are still winterly and the supply of light and nutrient salts is sufficient to cause a spring bloom of diatoms.

Observations on the occurrence of ceratia in Drøbak Sound (in the Oslofjord) show that some species were lacking periodically during the winter and spring 1950–51 (Table 2, p. 207). In this locality wind and currents sometimes sweep the surface layers out of the fjord and in this way remove a population with a low rate of reproductivity. It is less important in this case that the surface temperature goes down to 0° C, since, according to CLEVE's observations (1900), all the species are found outside the fjord and in the North Sea throughout the whole winter.

Growth rate experiments under different temperatures, the results of which are given in Tables XVII–XX, were carried out in thermostat boxes controlled with Sunvic thermostats, type TS1, with an accuracy of ± 0.5° C. Temperature adaption stock cultures for the steps used were obtained through successive transfers from stock cultures at room temperature. It was not possible, however, to obtain growth in experimental cultures at extreme temperature levels, even

when the cells survived in stock cultures. At 5° C all adaption cultures contained living cells, whereas only *C. tripos* and *C. lineatum* survived in experimental cultures. In this series of experiments the range of survival was not observed within as large a range as observed in nature. Further observations are given in the tables.

Ceratium tripos. The result of the experiment is given in Table XVII. Variance analyses gave a probability of 0.20 — 0.05 for treatment and > 0.20 for parallels.

C. fusus. Results given in Table XVIII. Probability < 0.001 for treatment and > 0.25 for parallels.

C. furca. Results given in Table XIX. Probability < 0.001 for treatment and 0.25 — 0.10 for parallels.

C. lineatum. Results given in Table XX. Probability 0.005 — 0.001 for treatment and 0.25 for parallels.

Discussion. The variance analyses showed that the number of parallels was sufficient for a dependable result. However the result obtained for *C. tripos* makes the dependability of the temperature curve questionable. This can be seen directly from Table XVII, where overlappings between single values in different temperature steps are found. This means that the difference in growth rate with the change of temperature is too small to outweigh the distribution within parallels. The other organisms show marked dependence of growth rate on temperature.

The results from the four temperature experiments are given graphically in Fig. 6.

From Table 2, p. 207 it can be seen that *Ceratium tripos* is the species found most frequently when sea temperature is low, and accordingly, it has a reproductivity which can partly compensate for the dilution of the population by transport due to currents. According to CLEVE (1900) *C. tripos* is common in the North Sea at temperatures down to 3.5° C. The growth ability of *C. lineatum* at 5° C, seen from Fig. 6, was not marked enough to have maintained a population in Drøbak Sound under the conditions found in the winter of 1950–51. That *C. lineatum* is not always absent from this locality at low temperatures is evident from the fact that the stock culture of this organism was isolated in January at 2° C (see p. 207).

The fact that high temperatures are beneficial for growth of ceratia under natural conditions was briefly touched upon in the introduction to this chapter. The temperature optima found in the experiments show that *C. lineatum*, appearing in Oslofjord as a cold water species has a higher temperature optimum than the other three species. However the optima given can not be regarded as absolute values since intermediate temperature intervals have not been investigated (Fig. 6). Observations show that *C. lineatum* is seldom found in the Oslofjord during the summer when the temperature exceeds 15° C

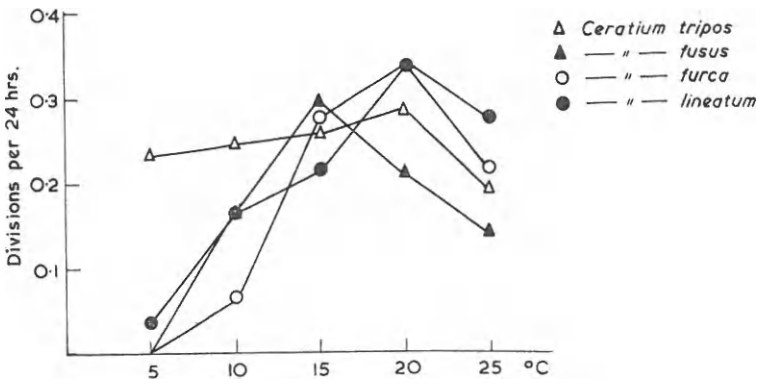


Fig. 6. Division rates at various temperatures.

(GAARDER and GRAN 1927, BRAARUD and BURSA 1939, BRAARUD 1945, BIRKENES and BRAARUD 1952). The absence of *C. lineatum* in this locality is not in accordance with its temperature optimum found in the experiment, but perhaps due to a combination of other environmental factors. GRAHAM and BRONIKOVSKY (1944) report finds of *C. lineatum* at temperatures up to 26.6°C.

The growth curves of *C. fusus* and *C. furca* are in good accordance with their occurrence in natural surroundings.

3. Light

Being photosynthetic organisms, ceratia require light for assimilation and growth. In addition, they react to light with phototactic movements, a factor which is responsible for their diurnal vertical migration (HASLE 1950), and which may be of value to their maintenance in the euphotic layer (PETERS 1929).

Since depth distribution of ceratia in the sea may partly depend upon the light requirement of the various species, it was of interest to determine whether *Ceratium* species in culture would show any optima and whether such optima would be specific.

Some preliminary experiments on the dependence of growth rate upon light intensity were performed. Two culture series of *Ceratium tripos* were placed in a north window. One of these series was shadowed with white paper to a light intensity three fourths of daylight. The absolute values cannot be given owing to the fluctuation of daylight. In the period 18 July – 1 August the organisms in the shadowed cultures showed an average division rate of 0.34 per 24 hours, while those in full daylight showed 0.29.

Since the optimal light intensity apparently was to be sought at a lower level than diffuse daylight, another experiment with more light intensity steps was started, now with *C. tripos* and *C. furca*. The light intensity steps were obtained

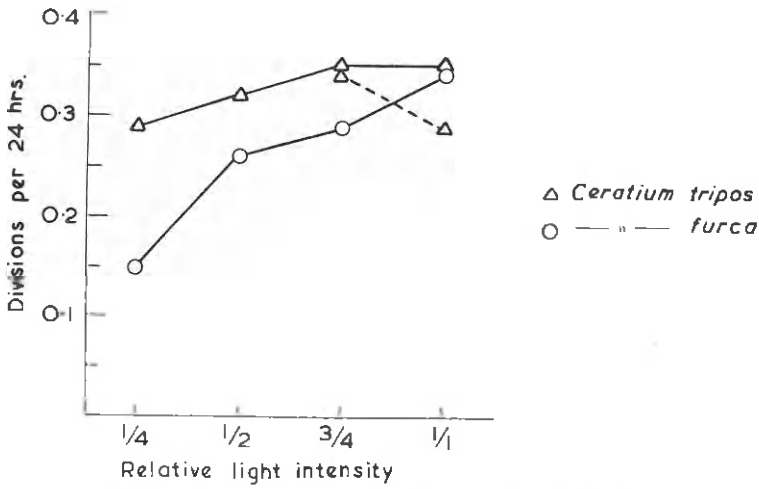


Fig. 7. Division rates in the preliminary experiments with varying light intensities.

in the same manner as mentioned above; the proportions were 1: 3/4: 1/2: 1/4. Results of the experiments are given in Tables XXI and XXII, which also include details on initial numbers, etc. In Fig. 7 the results are given graphically together with the two points from the first *C. tripos* experiment (see also Table II). The discrepancy between the results of the two *C. tripos* experiments was explained through differences in the light supply, indicated by meteorological data from Ås[1]), the observation post nearest to the Biological Station at Drøbak, where the experiments were performed. In the first period, 18 July – 1 August, the average daily cloud cover was 3.89 (scale 0–8), whereas during the second period, 8–20 August, in addition to a lower altitude of the sun, the cloud cover was 4.15. Overlapping of the cell numbers in the experiments at different light intensities made the results rather uncertain. The conclusions drawn from the preliminary experiments are as follows:

1. Light intensity influences the division rate.
2. The light intensity steps were too small.
3. Daylight is not a suitable light source in comparative quantitative experiments.

In view of the preliminary experiments, another series of growth experiments was performed, but using artificial illumination with light intensities of 10,000; 5,000; 2,500; 1,000 and 500 Lux, measurements made with a photo cell. The arrangement of cultures was as shown in Fig. 8. The experiments were carried

[1]) Placed at the author's disposal by the Meteorologisk institutt, Blindern.

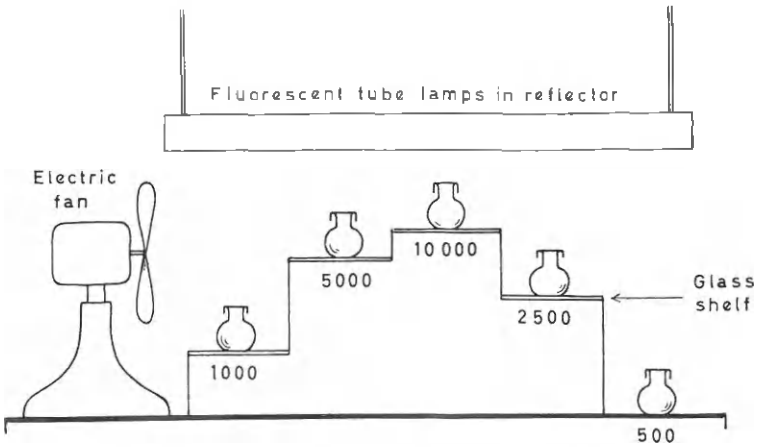


Fig. 8. Arrangement of the light intensity experiment. (Light levels given in Lux.)

out at room temperature and an electric fan used to provide an air current to prevent unequal heating of the cultures. Control flasks with thermometers were placed at each light level.

Ceratium tripos. The result of the experiment is given in Table XXIII. Probability ≈ 0.001 for treatment and > 0.25 for parallels.

C. fusus. Results given in Table XXIV. Probability < 0.001 for treatment and $0.10 - 0.05$ for parallels. Two experiments with this organism failed. Examination of the cultures revealed that the cells under the highest light intensities gathered in the surface film and died, probably trapped through phototactic movement. In order to prevent such trapping, the water surface had to be diminished, both absolutely and relatively. This was done by using 50 ml Erlenmeyer flasks filled to the neck as culture vessels. From earlier experiments it was known that *C. fusus* has a rather high mortality under transfer, so a diluted stock culture in good growth was used instead of a pipette inoculation of single cells. Control counts of three different 50 ml samples gave 34, 28 and 30 cells respectively, an average of 31 cells per culture flask.

C. furca. Results given in Table XXV. Probability < 0.001 for treatment and > 0.25 for parallels. In this and the following experiment diluted stock cultures were used instead of inoculation with single cells. The stock cultures were so dense that only small volumes were controlled, and both initial and end numbers are given in cells per ml. For *C. furca*, counting of three different 10 ml samples had 315, 312 and 333 cells, giving an average of 32 cells per ml.

C. lineatum. Results given in Table XXVI. Probability < 0.001 for treatment and $0.25 - 0.10$ for parallels. Three 10 ml control countings of the stock culture gave 652, 607 and 754 cells, an average of 70 cells per ml.

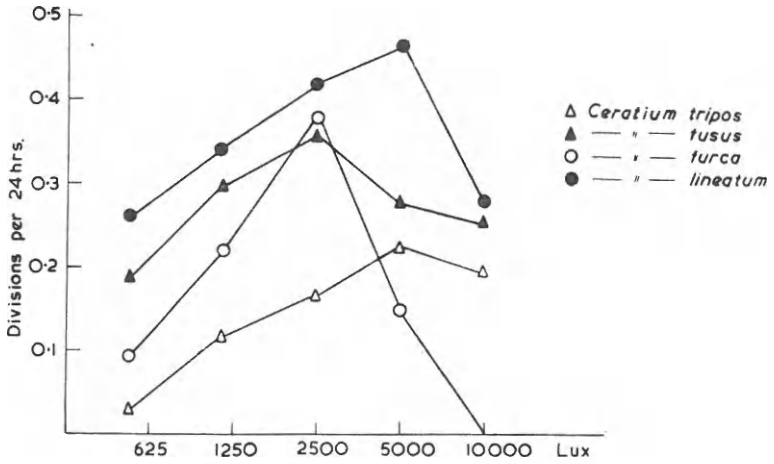


Fig. 9. Division rates at various light intensities.

Discussion. Values for division rates are given graphically in fig. 9. The Lux scale is logarithmic, and therefore points of 500 and 1000 Lux lie slightly to the left of the gauging points.

The most striking feature in the curves is that *Ceratium furca* would not grow at 10,000 Lux. If this were true under natural conditions in the sea, *C. furca* should not be found in the surface layers where light intensity on bright days often exceeds 50,000 Lux. However, it is found in maximal numbers in the upper few metres, and especially in summer when light intensity is high and temperatures exceed 20°C (Braarud and Bursa 1939, Braarud 1945, Hasle 1954).

C. fusus which, in the experiment, also showed decreasing division rate under light intensities higher than 2500 Lux, is found in high numbers in the surface layers of the sea. However investigations reveal that its maximum is not at the very surface and that its phototactic movement is pronounced (Hasle 1950). The discrepancy between the light tolerance in cultures and under natural conditions may be attributed to the continuous illumination in the cultures, the effect of which is known to be less favourable than diurnal illumination (Hasle and Nordli 1951).

An experiment on phototaxis made with the four *Ceratium* species showed that *C. furca* had the most rapid reaction to light, followed closely by *C. fusus* and in a much lesser degree by *C. lineatum*. *C. tripos* reacted very slowly. The arrangement of the experiment is as shown in Fig. 10. *C. furca* gathered near the light source within two days, and within a week died out, forming a clot of disintegrating cells in the bend of the tube. *C. fusus* had a distinct maximum at the same place within three days, but survived for several weeks. *C. lineatum*

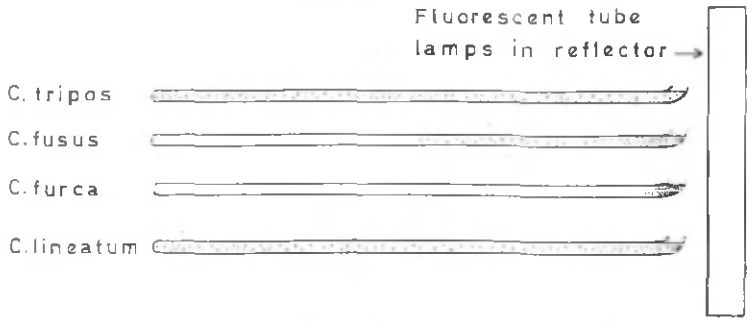


Fig. 10. Phototactic reactions in horizontal tubes.

also gathered in the half of the tube nearest to the light source. This was not visible to the naked eye, but could be seen through a hand lens. No aggregation toward light could be observed in *C. tripos*. The maximal light intensity of the experiment was 7000 Lux.

C. furca and *C. fusus* which, according to Fig. 9 have their light optima at approximately 2500 Lux, probably would have a higher division rate at higher light intensities if their marked phototaxis under culture conditions were not unfavourable. Difficulties owing to this were observed in the experiment with *C. fusus* (see p. 223). One unfavourable condition in the cultures may have been the continuous illumination which, except in very high latitudes, would never occur in a natural environment.

As a rule, plankton samples taken with water samplers and counted after sedimentation contain so few ceratia that the accumulation in the uppermost layers is not seen so clearly. Furthermore, the depths generally used for sampling are too far apart for this purpose. Some examples from the Oslofjord (BRAARUD and BURSA 1939, BRAARUD 1945, HASLE 1950 and 1954) are exceptions.

When net samples are taken, either with horizontal or vertical hauls, the depths are so far apart or divided in such high water columns that only distinctions between "surface" and "shade" species can be made (STEEMANN NIELSEN 1934, GRAHAM and BRONIKOVSKY 1944). The species dealt with in this paper were considered as surface species in both investigations mentioned. In tables compiled by APSTEIN (1905) vertical net hauls reveal that the uppermost five metres generally contain many more ceratia than any other layer, but this is also true in part for diatoms, the bulk of which are not provided with locomotory organs.

Observation material from natural surroundings is too scarce to provide a basis for a general discussion of the microstratification of the ceratia in question as a response to light intensity.

At an incident light of 100,000 Lux which is regarded an approximate maximum, the 1 % level is found to lie below the light optima for all ceratia tested. The position of this level in the sea depends upon transparency of the water. In habour water this level is found at a depth of less than 10 metres, in coastal water at approximately 25 metres, and in clear oceanic water at a depth of 150 metres (CLARKE 1933). The same value as for harbour water was found in the Oslofjord by HASLE (1950). These data taken into account, a pronounced microstratification of ceratia in the open ocean would not be expected, whereas such a stratification would be expected, and in fact is found, in coastal and inshore waters.

4. Nutrient salts and soil extract

The investigation was performed only with *Ceratium furca*, as this was the only species which showed sufficiently good growth in oceanic water to be used in comparative experiments.

An experiment series was performed with a constant amount of soil extract, 50 ml per litre, varying amounts of P + N of proportions as in Schreiber's solution, and ordinary Schreiber's solution with varying amounts of soil extract. The following set-up was used, each x representing five parallels, those with ordinary Schreiber's solution + 50 ml/l soil extract being identical in the two observation series.

Table 4.

Concentration of P+N	Concentration of soil extract			
	0	0.1	1	10
0			×	
0.1			×	
1	×	×	×	×
10			×	

The soil extract was adjusted with NaCl p. a. to the salinity of sea water so that addition of different volumes would not alter the salinity of the culture fluid. An initial number of ten cells in each culture vessel was used.

The results of the experiments are given in Table XXVII for varying amounts of P + N and in Table XXVIII for varying amounts of soil extract. Variance analyses gave probabilities of < 0.001 for both treatments and > 0.20 for parallels.

Since the experiment with different amounts of P + N revealed that the

supply of these elements from the sea water was sufficient, and that the addition of nutrient salts apparently did not affect the division rate, the next experiment for comparison with oceanic water was done with the usual amounts of P + N according to Schreiber's formula. This experiment was performed with sea water from the Oslofjord and from the oceanic Weather Ship Position "M" (66°N 2°E). The variable in the culture fluid was soil extract, besides the differences caused by the two sources of water, the effect of which could not be estimated. The results from the previous experiment (Table XXVIII) taken into account, the amount of soil extract was reduced and more steps included, forming a relative concentration series of 0: 0.2: 0.5: 1: 2: 4, where 50 ml/l = 1. In Tables XXIX and XXX the quantities are given in ml/l. Through addition of glass-distilled water the ocean water was adjusted to the salinity of the Oslofjord water. The soil extract was adjusted to the proper salinity before addition.

An initial number of 25 cells was used for each flask. The end number was determined by counting the cells in the total volume of the culture fluid.

The values for the growth in the Oslofjord water are given in Table XXIX. A variance analysis gave a probability of < 0.001 for treatment and > 0.25 for parallels. The values for growth in the ocean water are given in Table XXX. Probability: 0.005 — 0.001 for treatment, and 0.25 — 0.20 for parallels.

Discussion. The concentrations of P + N used by SCHREIBER (1927) did not give higher division rates in a nutrient solution prepared from Oslofjord water than in the same water with no addition of nutrient salts other than those possibly present in the soil extract. Ten times the amount used by Schreiber retarded the division rate significantly, indicating an overdosing. In view of the sewage contamination of the Oslofjord and the consequent abundant supply of nutrient salts (BRAARUD and RUUD 1937, BRAARUD and BURSA 1939, BRAARUD 1945), it appears that the concentration of P + N in the laboratory supply of aged sea water from the Oslofjord was sufficient to provide as good a growth as the culture conditions were able to render.

No investigation was made here concerning the composition of the soil extract used, since these experiments were to ascertain only whether soil extract can alter ocean water to a solution more like neritic water, which is known to contain a higher number of phytoplankton organisms. In the experiment, the results of which are given in Table XXXIII, the omittance of soil extract caused negative cultures whereas its addition gave more or less the same results as fjord water. Soil extract or any substitute for it, therefore, (NORDLI 1957) seems to be necessary to make ocean water suitable as a culture medium for *Ceratium furca*. Fjord water, and probably coastal water to a lesser degree, contains substances which, to a certain extent, permit the omission of soil extract. In general, however, such water does not seem to contain elements or compounds which can replace those contained in soil extract. A definite optimal

quantity of soil extract cannot be given since the sources of soil differ from batch to batch and, in part also in the method of preparation. In most cases, however, a reduction of the usual 50 ml/l by at least half seems advisable.

Ceratium tripos, *C. fusus* and *C. lineatum* did not grow in ocean water + soil extract with creditable enough results that growth curves could be calculated. Some of the cultures grew so well, however, that they supplied material for an investigation on the interdependence of length distribution and culture conditions, which will be described on the following pages.

C. The effect of environmental factors upon form variation

1. Length variation

An investigation of length distribution under various conditions was undertaken for *Ceratium fusus*, *C. furca* and *C. lineatum*, the length of which can be measured in a constant, well-defined way. Materials were supplied from experiments made for other purposes as well as from cultures grown especially for this analysis. Samples for comparison came from natural surroundings. Each length distribution diagram is based on 100 measurements, the values of which are subdivided into classes of 10 μ. The few exceptions are indicated.

a. Differences between cultures and natural populations. Cultures always seem to have shorter cells and a narrower range of length variation than samples from natural populations. Since material for the primary isolations originated from Drøbak Sound, material of natural population for comparison was also taken in this locality, and supplemented with control material from an oceanic station, Weather Ship Position "M" (66°N 2°E). Length measurements for *C. fusus* are given in Fig. 11. The two different culture clones gave approximately the same values, whereas the two natural populations formed their own individual diagrams, with a range of length variation approximately twice that found in cultures.

C. furca, Fig. 12, and *C. lineatum*, Fig. 13, gave measurements similar to those of *C. fusus*, namely, shorter cells in cultures than in natural populations. *C. fusus* and *C. furca* from natural populations had longer cells in oceanic than in neritic water.

In these investigations on the differences between natural populations and cultures, variation in temperature, division rates and population densities were not taken into consideration. The samples from natural surroundings were taken with a net; neither the densities of the populations, nor the division rates, therefore, are known. The temperature in the sea when the net samples were taken was about 12°C for *C. fusus* and *C. furca* and about 6°C for *C. lineatum*. All the cultures were grown at room temperature, 18–20°C.

b. The influence of temperature variations. In order to determine whether

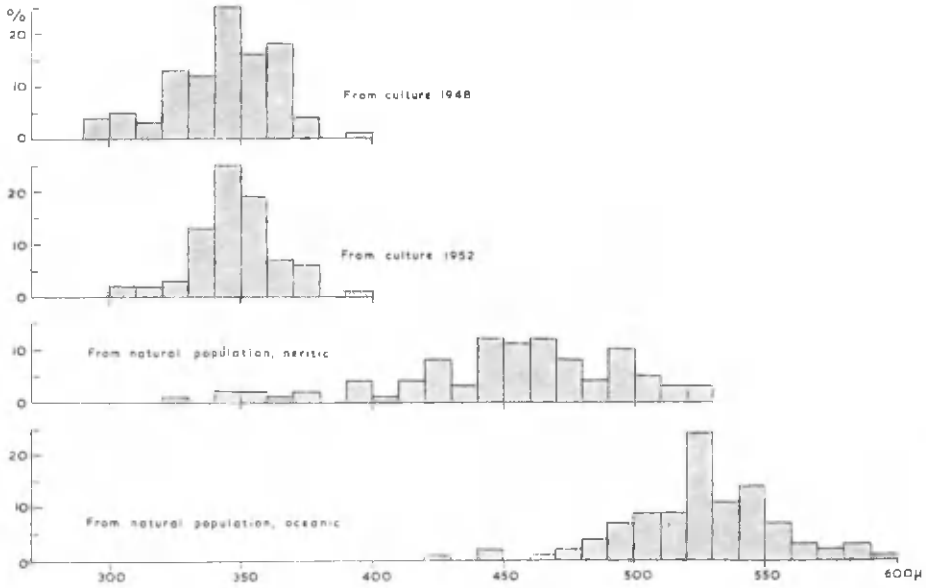


Fig. 11. Length distribution diagrams for *Ceratium fusus* from two clone cultures and two natural populations.

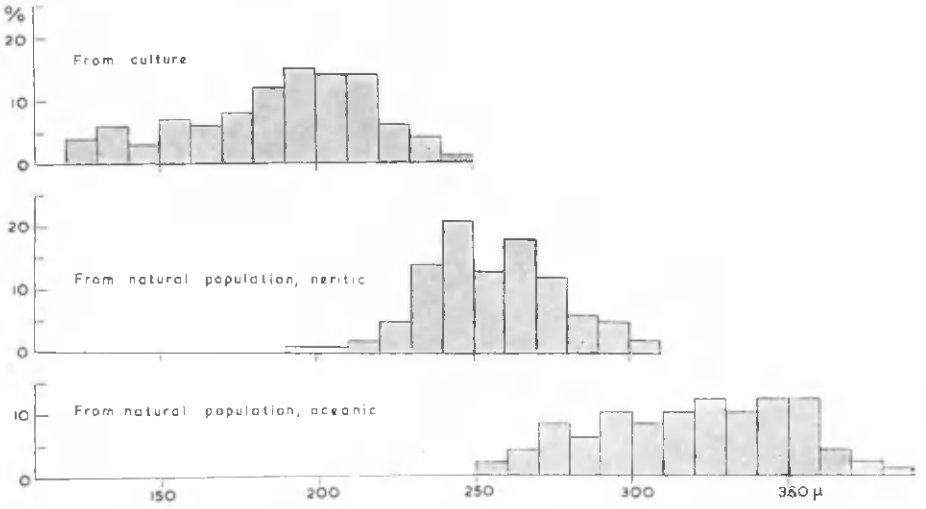


Fig. 12. Length distribution diagrams for *Ceratium furca* from one clone culture and two natural populations.

variations in temperature cause any alteration in length distribution of ceratia, representative samples from the temperature experiments given in Tables XVIII, XIX and XX were measured and length distribution diagrams constructed.

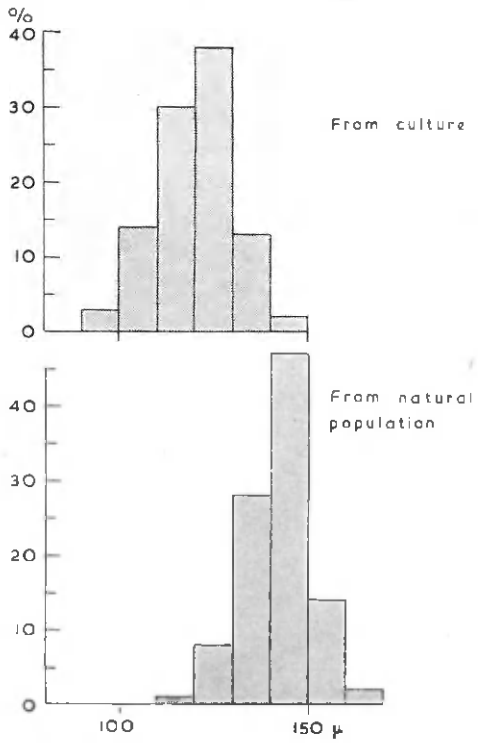


Fig. 13. Length distribution diagrams for *Ceratium lineatum* from clone culture and natural population.

Diagrams for length distribution of *C. fusus*, *C. furca* and *C. lineatum* under temperatures of 25°, 20°, 15°, and 10° C are given in Figs. 14 and 15. *C. fusus* and *C. lineatum* show increasing length with decreasing temperature, whereas *C. furca* does not show this tendency in any marked degree. A comparison between natural populations and cultures of *C. furca* revealed the same length diminishings under culture conditions as in the other two species. It appears, therefore, that temperature differences could not be the only cause for the different lengths found in the cultures and natural populations. That the division rates are not responsible for the length differences is evident from the fact that the average lengths decrease gradually from 25° C to 10° C, whereas the division rates have maxima at 15–20° C.

c. The influence of culture density. The length distribution in cultures of varying densities was investigated. All the cultures were started with a few cells and grown to the densities specified. Samples for measuring were taken out of the cultures during the culture period and therefore were not of the same densities for the different species. Even the thinnest culture had a lower average cell length than any found in natural populations where densities are often

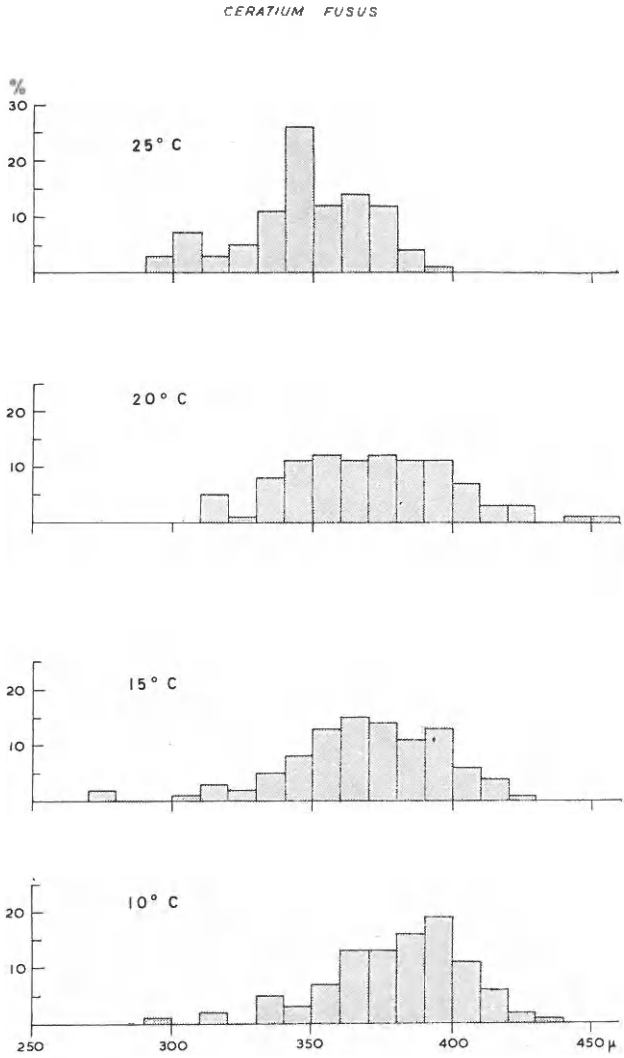


Fig. 14. Length distribution diagrams for *Ceratium fusus* grown under various temperatures.

proportionate to the lowest culture values (HASLE 1950 and 1954, NORDLI 1951). In the experiments the cell lengths of all the organisms decreased with increasing culture densities. Diagrams, with the culture densities, are given in Fig. 16.

d. The influence of water sources. A comparison of the length distributions of the three species grown in culture media prepared from different sources of sea water was undertaken. The two water types were neritic water from the Oslo-

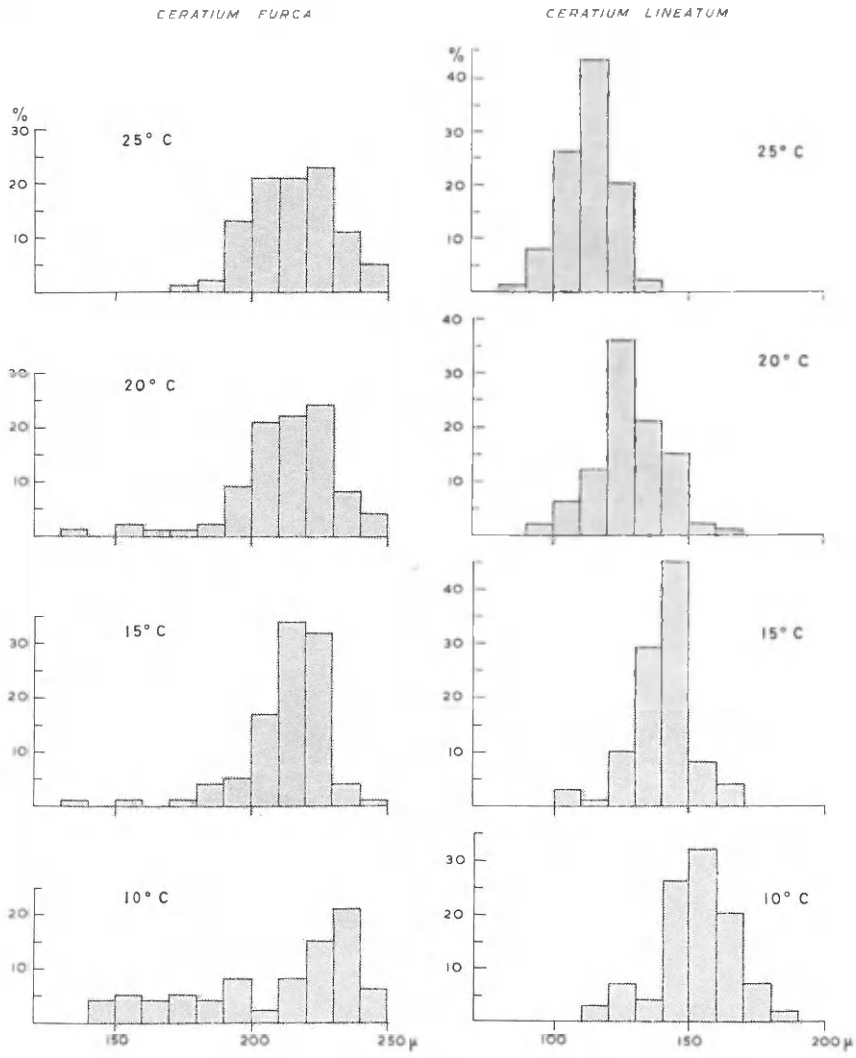


Fig. 15. Length distribution diagrams for *Ceratium furca* and *C. lineatum* grown under various temperatures.

fjord and ocean water from Weather Ship Position "M" (66°N 2°E). The ocean water was adjusted to the same salinity as the neritic water. From the first type "Erdschreiber" was prepared, and from the second ordinary Schreiber's solution without soil extract. All cultures were grown at identical temperature and light conditions, 18°C and 1500 Lux. Diagrams for length distribution of the three species in the two nutrient solutions (Fig. 17) are based on measurements of 200 cells for each diagram.

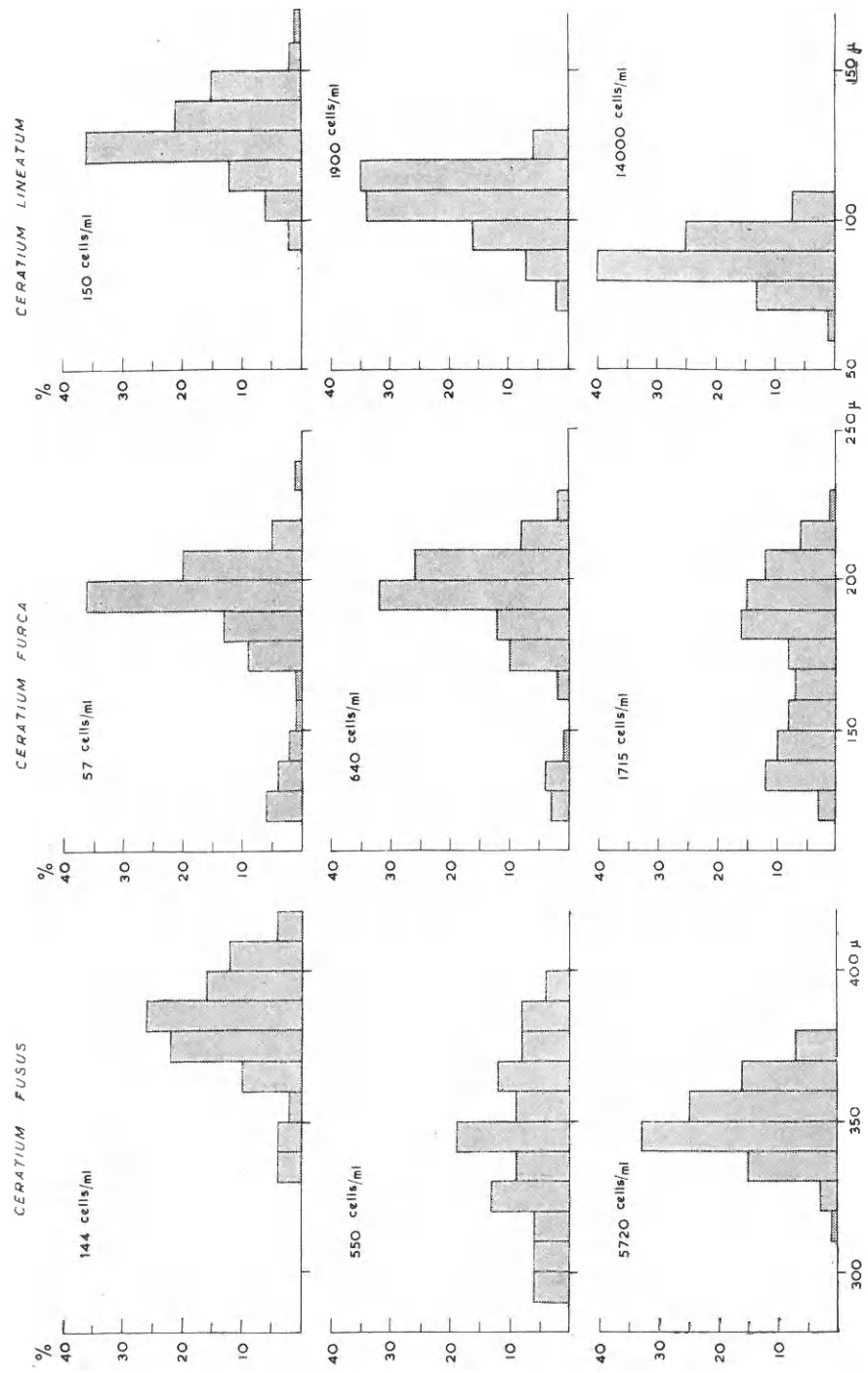


Fig. 16. Length distribution diagrams for *Ceratium fusus*, *C. furca* and *C. lineatum* from various culture densities.

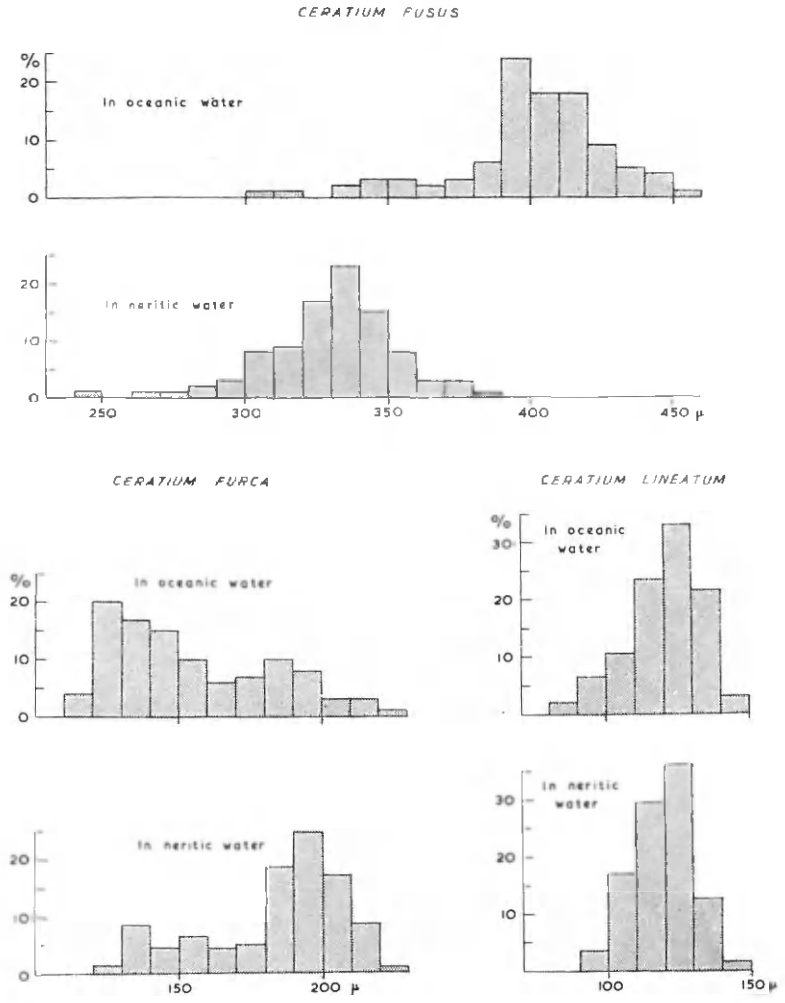


Fig. 17. Length distribution diagrams for *Ceratium fusus*, *C. furca* and *C. lineatum* grown in nutrient solutions prepared from different sea water sources.

Discussion. From the investigation on length distribution some rather well-founded conclusions can be drawn:

1. The average cell length is shorter in cultures than in natural populations, but this difference can not be ascribed solely to the different temperatures to which these two population types commonly are subjected. Measurements of *Ceratium fusus*, the largest of the organisms in question, revealed a marked difference in length distribution at identical temperatures in the two biotopes. In *C. furca* and *C. lineatum* the relationship is more obscure, perhaps because of the inefficiency of the method of distinguishing temperature effects from other

effects, or because the observation material was too scanty and the measuring method for such small organisms is rather rough.

2. Cell lengths decrease with increasing culture density. As the cultures employed were not bacteria-free, and as the division rates of bacteria are much more rapid than the division rates of the organisms investigated, the old dense cultures were more contaminated than the fresh ones. Such contamination might have been responsible for the decrease in length of the old culture cells. The accumulation of metabolites from the test organisms themselves also might have been responsible, but how these substances act is rather obscure at present despite the numerous observations concerning the problem. (For literature, see LUCAS 1947.)

3. With increasing temperature cell lengths decrease pronouncedly for *C. fusus* and *C. lineatum*, and in a lesser degree for *C. furca*. That division rate is not responsible for this appears from the fact that the cultures with a low division rate at 25° C produce short cells; those at 10° C produce long cells, while those with higher division rates produce cells of intermediate lengths when at intermediate temperatures. The consequence of bacterial growth, which is more rapid at higher temperatures, should not be overlooked.

The experiment comparing oceanic and neritic water did not render any information as to the reason for the length differences in the two types of water. Culture density at first might be assumed responsible in the case of *C. fusus* and *C. lineatum*, but the measuring results for *C. furca* contradict such a view. Probably a complex of interacting factors not distinguished by the investigation method is responsible.

Various authors have demonstrated that cell lengths may vary among localities. STEEMANN NIELSEN (1934) found that *C. fusus* from oceanic stations showed a shorter average length than cells from neritic stations. The measurements given in Fig. 11 where cells from neritic water are found to be shorter than those from oceanic water do not corroborate Steemann Nielsen's findings. If Steemann Nielsen's measurements are extensive enough to justify the conclusion that *Ceratium* specimens from neritic waters are longer than those from oceanic waters, the present experiment should indicate that in constitution water from the Oslofjord is more closely related to "Erdschreiber" than to ordinary neritic water. This may be due to an abundant supply of nutrient salts and other substances originating from sewage supply in the innermost part of the fjord.

Measurements of *C. fusus* from the oceanic Weather Ship Position "M" display a length distribution similar to that found by STEEMANN NIELSEN (1934) at 26° C at St. 1213 in the Gulf of Panama where he recorded the greatest average length of his samples. The average length of *C. fusus* from the coastal waters from Hong Kong to Shanghai (temperature 22.5° — 26.9° C given by BÖHM 1931 b) are similar to those found in the Oslofjord at 10° C.

C. furca from Position "M" showed a greater average length than that registered by STEEMANN NIELSEN (1934), and was approximately 100 μ longer than the large form reported by BÖHM (1931b). *C. furca* from the Oslofjord also had a greater average length than Böhm's large form. From the sample collected at Position "M" only 50 *C. furca* cells were measured, owing to the scantiness of material. The sample contained too few *C. lineatum* specimens to form a basis for a dependable length distribution series.

From the sparse material available for comparison, it appears that *C. fusus* and *C. furca* found in the Norwegian Sea and the Oslofjord display greater average lengths than the same species in warmer seas. This may be due to temperature and other environmental conditions, or to genotypical variations of the species. The *C. furca* specimens collected at Position "M" may belong to the subspecies *Berghii*, whereas those from the Oslofjord may be subspecies *eugrammum*. According to JØRGENSEN (1911) the latter should be rather common in all warmer seas, but in small numbers. In the Oslofjord, however, this subspecies is found in dense populations. It has not been possible to cultivate either of the two species from oceanic waters. A comparison of the culture material of oceanic and neritic forms of these two species, therefore, has not been possible.

It may not be incorrect to ascribe length variations in these *Ceratium* species to differences in genetic factors, as BÖHM does (1931 b) for *C. furca*, but present knowledge of environmental influences seems to make such an interpretation questionable.

2. *Other form variations*

Ever since *Ceratium tripos* was described by O. F. MÜLLER (1776) under the name *Cercaria tripos*, and SCHRANK (1793) gave the name *Ceratium* to the genus, a large number of species have been described. JØRGENSEN (1911), in his monography on the genus *Ceratium* compiled a systematic description which has not been essentially altered since.

As this paper deals with only four species of the genus, no general discussion on systematic position will be presented here. With a few exceptions, only material from clone cultures will be treated, and examples of the variability within such uniform material will be shown.

a. Variability in "normal" cells.[1]) *Ceratium tripos.* The clone culture was started from a *C. tripos* f. *atlantica* Ostenfeld specimen, so the majority of the

[1]) A cell is regarded as "normal" when the right number of horns is present and the different parts of the cell, horns, thecae etc. bear the characteristics of the species, regardless of measurements, proportions and horn directions.

cells in all the subcultures possess characteristics of this form (Pl. I*i*). Some of the organisms, however, deviate rather considerably from f. *atlantica*, and, though they cannot be classified as other forms, exhibit characteristics resembling other forms or subspecies. Pl. I *a*, *d*, *f*, and *h* illustrate some form variations; *a* shows features in common with subsp. *balticum* Schütt, and would probably be best classified as an intermediate form between f. *atlantica* and subsp. *balticum*. This form was not uncommon in the cultures; *d*, *f* and *h* show variations in the directions and bending of hind horns; *d* resembles *C. leptosomum* Jørgensen (1911 Fig. 167) in form and direction of horns. The horn lengths, however, are typical of *C. tripos*. A certain resemblance to a form described by BÖHM (1931 c Fig. 26) is also present. Böhm, however, does not decide whether this specimen should be considered deformed. *d* also displays some features in common with *C. batavum* Paulsen (1907 Figs. 33a and b). JØRGENSEN (1911) will not accept the latter as a species, and supposes that the specimens are deviating forms of *C. intermedium* and *C. longipes*. No forms similar to *f* have been described from natural surroundings. Hind horns resembling *C. bucephalum* are found in the specimen shown in *h*. The form of the hypotheca, however, is that of *C. tripos*.

The small cells in Pl. I *i* are *truncata* forms of *C. tripos*. They commonly occur in dense cultures and, when isolated, will survive for a period up to a month long. Little is known of their significance in the life cycle of *C. tripos*. A discussion of their occurrence in cultures has been given by HASLE and NORDLI (1951).

C. fusus, *C. furca* and *C. lineatum*. The form variations of "normal" cells in these species are limited to variations in length and transdiameter. In Pl. II*b* different transdiameters with equal cell lengths are shown for *C. fusus*; II*a* shows the same for *C. lineatum*. It is easily seen from Pl. II*e* that size differences are not due to incomplete growth after fission since the epitheca as well as the hypotheca and transdiameter in the small cell are all proportionally smaller than in the large cell. An incomplete restoration after fission would have diminished the length of either the epitheca or the hypotheca.

b. Supernumerary horns. Cells with supernumerary horns are not rare in cultures. In most cases one horn is duplicated, but apart from this the cells appeared quite normal and had motility like normal cells.

In *Ceratium tripos* cultures the commonest duplication is of the left hind horn (Pl. I*g*). In an adaption culture at 25° C this form was found in a ratio of about 20 %. This culture was the healthiest looking and the quickest growing of five parallels, though it was not dense (429 cells in 30 ml). The duplication seemed to be effected by a splitting of the horn. Similar cells have been reported from natural surroundings (GOUGH 1905, Fig. 2; HASLE and NORDLI 1951 Fig. 4a). Where the right hind horn was duplicated, two distinct horns were

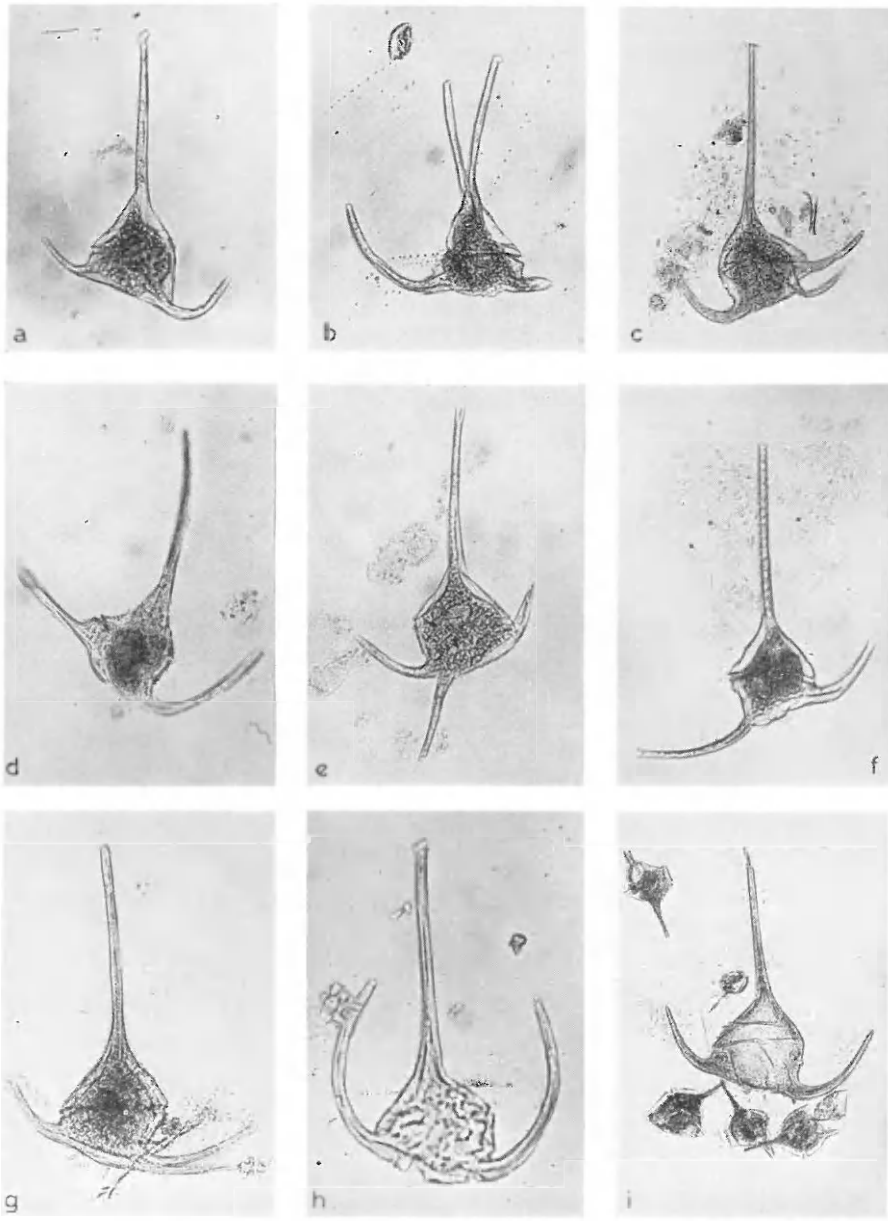


Plate I. Variability in *Ceratium tripos* cells (a–i).

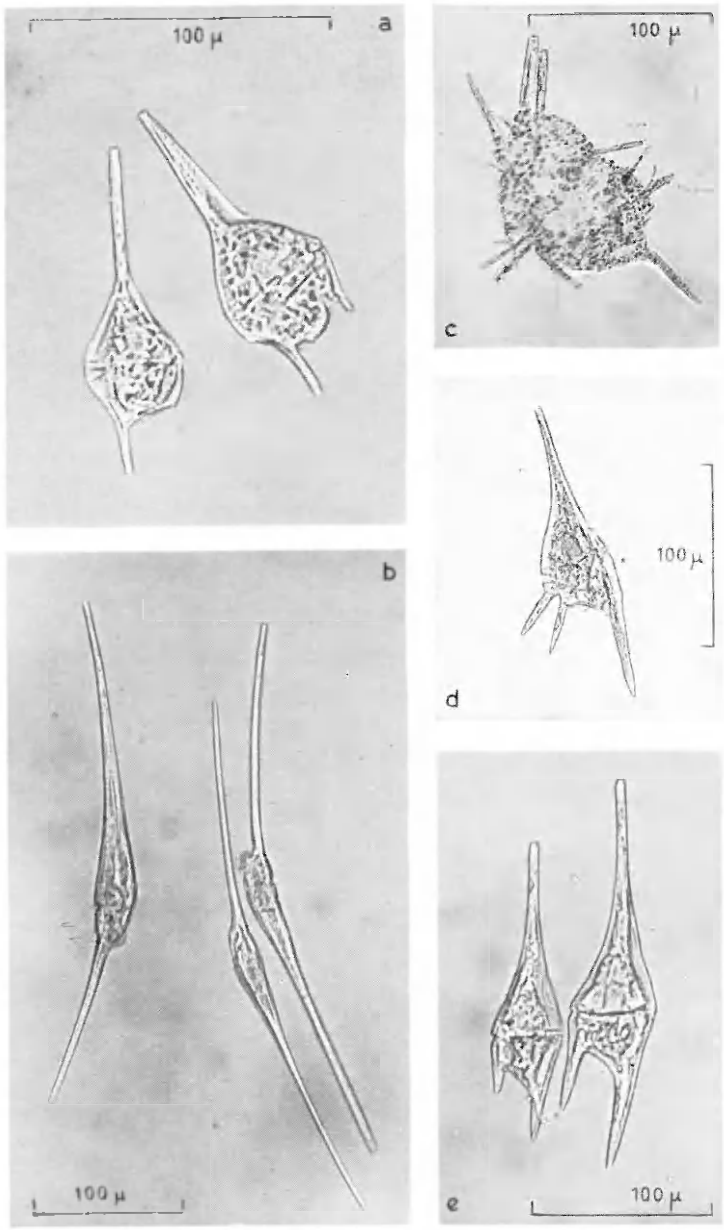


Plate II. Variability in *Ceratium fusus* (b), *C. furca* (d and e) and *C. lineatum* (a and c) cells.

observed. (Pl. I*c*). This type has also been reported from natural surroundings (HASLE and NORDLI 1951 Fig. 4b). One form, the parallel of which has not been observed in the sea, is that shown in Pl. I*b*. Whether the apical horn was split or two distinct horns were present is not known.

Occasionally two fully developed hind horns were found on *C. fusus*. When more than the ordinary number of horns was present, the cells were usually deformed in other ways too. Such forms will be treated later.

Supernumerary horns on *C. furca* were seldom found either in culture or in the sea (Pl. II*d*. HASLE and NORDLI 1951, p. 12, note).

In *C. lineatum* supernumerary horns were never observed apart from on heavily deformed cells. When the cell bodies were deformed beyond recognition, they often had excessive horns. In such cases the structure of plates on the cell body *proper* was indistinct; sometimes the cell seemed to lack plates completely, forming just a lump of protoplasm. These cells did not move in the ordinary way, as did the otherwise normal appearing cells having an extra horn. The heavily deformed cells sunk to the bottom of the culture flask and moved only occasionally. These abnormal forms were not uncommon in *C. tripos* and *C. fusus* cultures, but were rare in *C. lineatum* cultures and never observed in *C. furca* cultures.

Stainings with Belling's method were made in order to examine the nuclear conditions in the deformed cells. This method gives only the form and size of the nuclei present. In the deformed cells the form (Fig. 18) and number (Fig. 19) of the nuclei were abnormal. A normal *Ceratium* cell contains one more or less globular nucleus in the resting stage; after nuclear fission, but before cell fission, two nuclei are present. Contrary to this, the *C. fusus* cell in Fig. 19 divided even though the daughter cells would not part until new horns were partially grown out. *C. lineatum* (Pl. II*c*) was not subjected to nuclear staining since it was found in material already preserved with an agent unsuitable for the staining method. In cells with supernumerary horns but otherwise normal in appearance, the nuclei were as in normal cells.

Discussion. In phytoplankton material from various areas, cells with deviating forms are occasionally found. Forms similar to some of these were observed in cultures, and culture conditions may to some extent give a clue to the cause of their occurrences.

HASLE and NORDLI (1951) describe deviating forms of *Ceratium tripos* and *C. fusus*, suggesting that cells with supernumerary horns plus an otherwise more or less disordered form, may be due to a failing in the mechanism of cell division, caused by any unfavourable condition in cultures or heavily contaminated natural surroundings. According to the results obtained by nuclear staining of the disordered cells, it seems probable that deformations orginate from faults in the nuclear division mechanism, and that further growth is controlled by abnormally constituted or abnormal numbers of nuclei. KALLIO

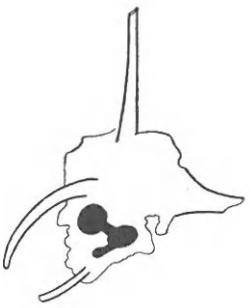


Fig. 18. Abnormal *Ceratium tripos* cell with abnormal nucleus.

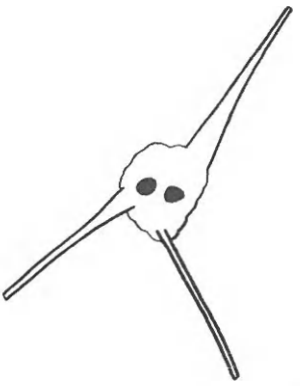


Fig. 19. Three-horned *Ceratium fusus* cell with two nuclei.

(1951) reports alterations in the shape of *Micrasterias* cells resulting from alteration in the nuclear material. Some of his abberant forms were results of temperature shocks.

The normal period for *Ceratium* cell division is during the night, consequently in darkness (BERGH 1886, GOUGH 1905, APSTEIN 1910). Cultures, however, are commonly reared under constant illumination. Since abberant cells are found more frequently in such cultures than in cultures grown under diurnal illumination and in plankton samples from the sea, it seems to be obvious that carbon dioxide assimilation during the period of nuclear fission produces disorder in its natural progress (HASLE and NORDLI 1951).

Influences different in nature and/or intensity from those found in the sea are bound to be present in cultures. Such influences may be due partly to the culture methods commonly used, the high concentration of nutrient salts, the increasing amount of bacteria and metabolites, and cell densities up to 1000 times those commonly found in natural surroundings. In some cases, as in the experiments with varying salinities, extreme influences are intended; deviating forms occur more frequently under such circumstances than in ordinary stock cultures (p. 237).

Deviating forms within clonal cultures, which genetically are uniform, show that a certain variability within a species is always present, and that the borders between natural variants and abnormal forms are indistinct. In investigations of natural populations this form variability must be kept under consideration to prevent borders of taxonomic units from being too narrowly drawn.

D. Observations on luminescence

With the exception of *Noctiluca*, where light is emitted from scattered granules within the cytoplasm, nothing is known about the mechanism of light

production in dinoflagellates. It is supposed, however, that in regard to phosphorescence the smaller dinoflagellates are organized in the same way as *Noctiluca* (HARVEY 1952, p. 130).

The luminescence of ceratia in cultures has never been subject to investigation, although net samples of these organisms were used in experiments on luminescence more than half a century ago (REINKE 1898). This may be because cultures of these organisms are required if a thorough study of dinoflagellate luminescence is to be undertaken (HARVEY 1952, p. 124).

Among the light producing protists in the sea *Ceratium tripos* is known to be a predominant species. Some cultures of this species being on hand, a few experiments to test its luminescent ability were performed. Since light emission from luminescent organisms takes place through oxidation of luciferin, the tests were made with oxygen – deficient material by bubbling air through cultures which had stood in darkness for a sufficient time, usually over night. It is possible that the maximal intensity of luminescence passed during the dark period used. HAXO and SWEENEY (1955) found in experiments with *Goniaulax polyedra* that the maximal capacity for luminescence was reached after a dark period of 6–8 hours. Afterwards the light emission decreased.

The culture to be tested was placed in a burette, the lower end of which was connected to a rubber tube leading from a small aquarium pump for air supply. A screw clip was attached to the tube for air regulation. The apparatus can be seen in Fig. 20. Tests were made in complete darkness, and only visual control was used.

C. tripos, *C. fusus*, *C. furca* and *C. lineatum* were tested, and except for *C. fusus*, no light emission was found. Since the *C. fusus* culture was the densest, containing approximately one million cells per litre, it was assumed that any phosphorescence from the other cultures was probably too faint to be seen. The experiment was repeated when the cultures grew dense, containing two to four million cells per litre, but gave the same negative result.

The question arose as to whether the *C. fusus* cells themselves were phosphorescent or whether the light came from light bacteria contained in the culture fluid. Another *C. fusus* culture, still luminescent when tested, was filtered through a G 3 Jena glass sintered filter with pores of 15 — 40 μ in which ceratia would be retained while the bacteria passed through. The filtrate was tested in the usual manner, with a negative result. If light bacteria were responsible for the luminescence, they must have been symbiotic and non-luminescent when separate from the *Ceratium* cells.

Cultures of *C. tripos*, *C. furca* and *C. lineatum* were injected with a few ml of the filtered culture fluid from the luminescent *C. fusus* culture. After this treatment, and rearing the cultures to a proper density, the three non-luminescent species were again tested in the usual manner, giving the same negative result as

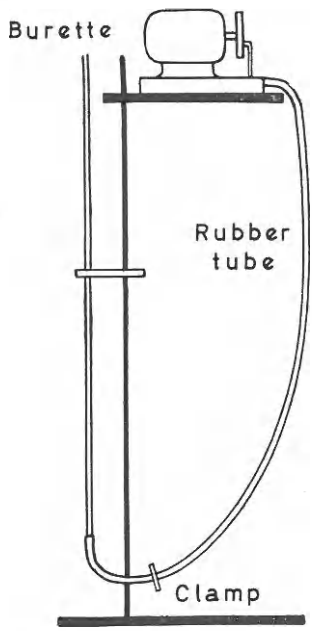


Fig. 20. Apparatus for testing of luminescent abilities.

before. If light bacteria were responsible, it would be expected that *C. tripos* at least, which is known to be phosphorescent in the sea, would have acquired ability to emit light. Light bacteria in such a case would be attached to the surface of the flagellates, since dinoflagellate luminescence originating from cytoplasm is known to be non-bacterial. Therefore the possibility that the luminescence in the *C. fusus* culture was due to light bacteria probably can be excluded.

The three *Ceratium* species which did not show luminescence were tested by some of the methods described by REINKE (1898), viz. ethanol, I–KI and Direct Current. The test with electricity was performed in the apparatus shown in Fig. 20, with the rubber tube detached and the electrodes placed in either end of the burette. The current was supplied from a 4.5 V and a 22.5 V dry battery. The other tests were performed in test-tubes. All the experiments employed cells taken directly from stock cultures as well as from cultures which had been subjected to a dark period. Several concentrations of chemicals were used. No luminescence was found in any of the experiments.

Since it was off season for ceratia in the sea when these experiments were performed, the methods could not be tried on natural plankton. REINKE (1898) used net samples diluted with sea water to a brownish colour, and his *Ceratium* samples probably contained many times the number of cells used in the experiments described here.

It is likely that the *C. fusus* culture possessed the same phosphorescent ability exhibited by ceratia in the sea, and that the other three cultures were non-luminescent clones. Unfortunately the luminescent *C. fusus* culture died out through an accident so further experiments could not be undertaken.

IV. Actual seasonal occurrence and geographical distribution compared with the experimental results

In a comparison between the experimental results and the geographical distribution of the ceratia it should always be kept in mind that the experiments are performed with clone cultures. Natural populations are not of such genetic uniformity, and even different subspecies may be present in the material with which comparisons are undertaken. The reliability of the results have to be regarded accordingly.

In previous chapters (pp. 217–226) it is shown that the four species *Ceratium tripos*, *C. fusus*, *C. furca* and *C. lineatum* react to variations in salinity, temperature and light intensity by changing their division rates. The beneficial influence of neritic water upon growth of phytoplankton organisms is discussed on p. 227.

Fig. 5 shows that the optimal salinities for the *Ceratium* species in question lie far below those commonly found in the sea. Fig. 6 shows that temperature optima for the same species lie at a level which in high latitudes is found only in restricted areas.

The annual amplitude of surface temperature and salinity is relatively large along the Norwegian coast and in the Transition Area[1]) where summer temperatures reach a maximum of 18–20°C and salinity a minimum of less than $18^0/_{00}$. At the entrance to the Baltic the salinity may go down to $7^0/_{00}$. The maps in Figs. 21 and 22 give the average August surface isotherms and isohalines for the southern part of these areas. (Maps based on various sources, mainly: "Atlas de température et salinité . . ." 1933.)

1. The distribution of ceratia in relation to salinity distribution in the Baltic

From Fig. 5 it can be seen that *Ceratium tripos* has considerable growth at a salinity of $10^0/_{00}$ where the other species in question will not grow. Corresponding to this, in summer *C. tripos* is commonly recorded eastward to Rügen, and small numbers are found farther east (Apstein 1905). *C. fusus*, *C. furca* and

[1]) Name adopted by "The International Council for the Exploration of the Sea" for the Danish sounds and adjacent waters.

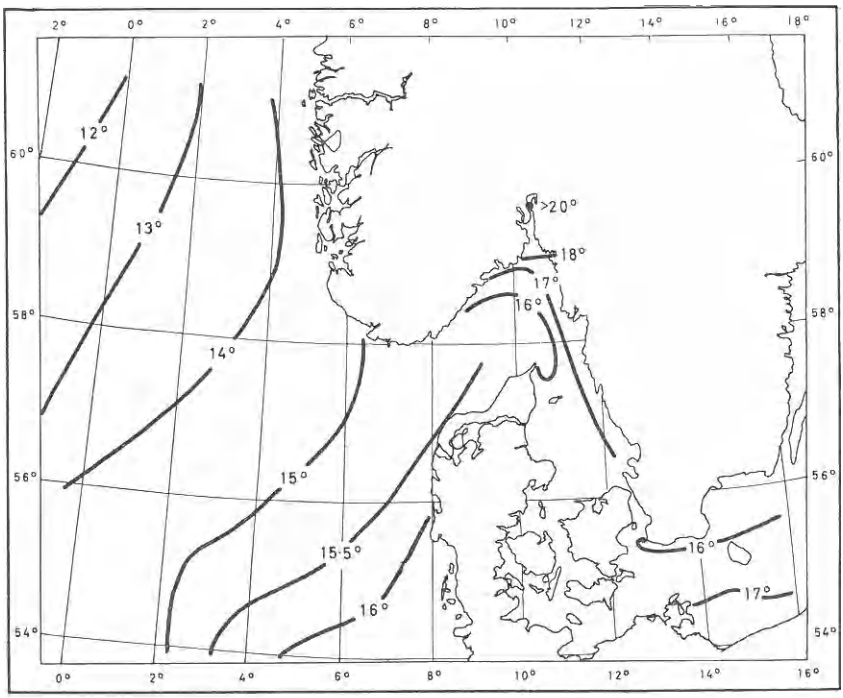


Fig. 21. Average August surface isotherms.

C. lineatum are seldom found in either of these areas when salinity is at its minimum. STEEMANN NIELSEN (1940, Tab. 17–22) recorded no ceratia where salinities only occasionally exceeded $10^0/_{00}$. AURIVILLIUS (1896) mentions that *C. fusus* decreases more rapidly eastward in the Baltic than *C. tripos* does. *C. fusus*, as well as *C. furca*, are never recorded as far eastward as *C. tripos*. It appears, therefore, that the tolerance for low salinity found in the experiment corresponds very well to the power of invading brackish water which has been displayed by these three species. Reactions to temperature have not been taken into account since data for comparison are too sparse.

2. The Transition Area

South of the Danish islands ceratia are abundant in summer at low salinities and medium to high temperatures, the populations remaining at high levels when the temperature decreases in autumn. Near Kiel (November 1, 1905) LOHMANN (1908, p. 276) recorded *C. tripos* in a number of 13,000 cells per litre. In the Transition Area BROCH (1909) found that *C. tripos* has a growth optimum in summer at approximately $20^0/_{00}$ S and high water temperature, and probably another optimum at approximately $30^0/_{00}$ S and low temperature. This second

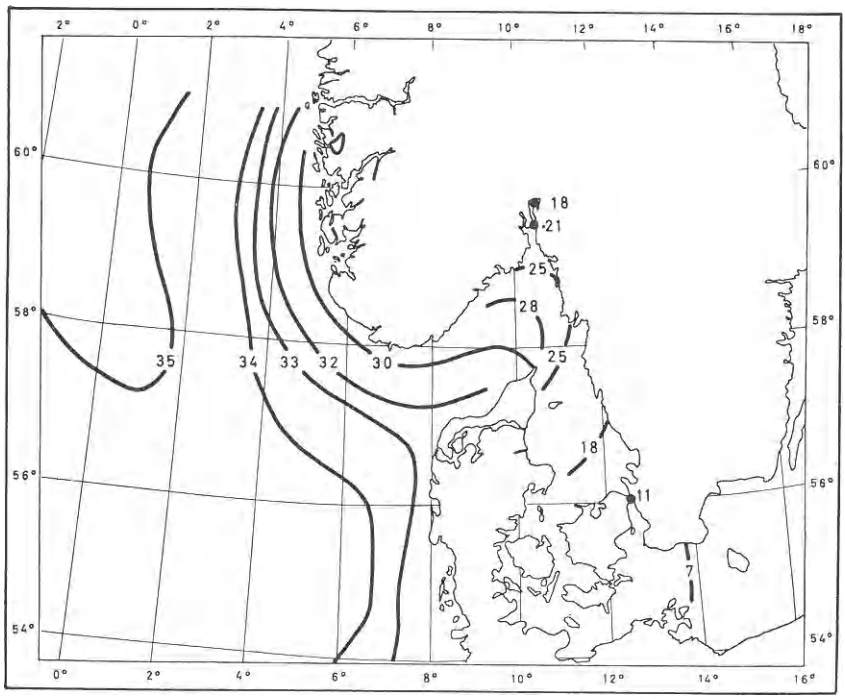


Fig. 22. Average August surface isohalines.

optimum might be responsible for the relatively high cell number in the deep layers during the summer. This high salinity population may replace the low salinity one when hydrographic conditions become wintry. APSTEIN (1910) reports maximal numbers of *C. tripos* and *C. fusus* in samples from Kieler Bucht (34° 30½′ N, 10° 21′ E) in autumn, salinity and temperature not given. OSTENFELD (1913) found that the occurrence of ceratia in Danish waters shows a yearly cycle with a maximum in late summer and autumn, when especially *C. tripos* can be a dominant species in the phytoplankton. In late winter and spring the ceratia are very rare or lacking. In Kieler Föhrde, TSCHIRN (1920) found that *C. tripos* and, to a certain degree, *C. fusus* were abundant. WATTENBERG and MEYER (1936) recorded dense populations of ceratia (species not specified) in autumn at salinities ranging from $10^0/_{00}$ to $18^0/_{00}$. In the Transition Area STEEMANN NIELSEN (1940, Tables 5–16) commonly found ceratia in considerable numbers at salinities ranging from $11^0/_{00}$ to $30^0/_{00}$ or more. Interpretation of the importance of the temperature data is complicated due to the special hydrographic conditions in the area. As a rule, however, the greatest numbers of cells are observed at relatively high temperatures and at medium salinities, with maximal numbers at 4200 cells per litre, species not specified.

3. The Oslofjord

In the Oslofjord ceratia are found in immense numbers during the summer when salinity is low and temperature high, sometimes in populations so dense as to cause "red water". Such a case was reported where *C. furca* and *Goniaulax polyedra* made up the majority of the plankton with numbers of 171,000 and 159,000 cells per litre respectively (NORDLI 1951). Owing to differences in size between these two organisms, *C. furca* must be the dominant species in colouring the water. In the innermost part of the fjord the water is often coloured by phytoplankton organisms, but smaller dinoflagellates outnumber the ceratia by many times. The number of ceratia, however, is so high (*C. tripos* exceeding 33,000, *C. fusus* 100,000 and *C. furca* 10,000 cells per litre HASLE 1954, NORDLI 1953) that these species might be responsible for a considerable part of the water colouring. Such high numbers of ceratia in this locality are not only found in observations of recent date. GRAN (1915) reported a collection of 13,860 cells per litre of *C. tripos* on October 20th, 1907. Since the order of magnitude was the same as the most recent observations to date, the difference in number might be ascribed to local variations. On the other hand, in view of the increasing contamination of the fjord from sewage (BRAARUD and RUUD 1937, BRAARUD 1945) there might be a real increase in cell numbers as well.

4. Skagerrak and the North Sea

In Skagerrak in the period March-June 1912, GRAN (1915) found increasing numbers of *C. tripos* and *C. fusus* with increasing temperature and decreasing salinity. A similar increase in the number of ceratia was also found in June in the station series from the Scottish coast to the vicinity of the Norwegian coast, where the numbers again decreased when the surface layers became unstable.

5. The waters off the Norwegian west coast

GRAN (1902) subdivided the Norwegian Sea and adjacent waters into different plankton regions: The area along the Norwegian west coast, according to him, forms the "Tripos-Region" and is characterized by temperate Atlantic-Oceanic plankton elements, among which *C. macroceros*, *C. tripos* and *C. fusus* are leading species. Calculations of mean summer surface temperatures and salinities reveal that this region is more or less bordered on the west by the $35^0/_{00}$ isohaline and the 12°C isotherm, with warmer and less saline water nearer the Norwegian coast.

In a later investigation outside the Romsdalsfjord GRAN (1928) found an increase of *C. tripos* from March to June. The average number of cells per litre was 5 in March, 51 in June. This increase in number was ascribed to a temper-

ature increase of approximately 4°C for the surface water in the outer stations. At the same stations the decrease in surface salinity was 1.65‰.

In all-year observations at four stations off the Norwegian west coast, BRAARUD, GAARDER and NORDLI (1957) found ceratia at varying temperatures and salinities throughout the year, with maxima in summer and autumn at relatively high temperatures. The salinity ranged from 20‰ to 34‰ with the commonest values 30–32‰. Maximal values per litre were 300 for *C. tripos*, 600 for *C. fusus*, 1200 for *C. furca* and 920 for *C. lineatum*.

6. Oceanic areas

Quantitative values for ceratia from the open ocean are scarce. For comparison with the numbers given above for coastal waters, the following data are given from two oceanic stations. In the Faroe-Shetland Channel (June 1912) the number of ceratia was small (20 cells per litre) and in most samples no ceratia were present (GRAN 1915, Table VII). In his all-year phytoplankton investigation from Weather Ship Position "M" (66° N 2° E) HALLDAL (1953) found ceratia in small numbers with 80 *C. tripos* and *C. fusus* and 40 *C. furca* and *C. lineatum* cells per litre as maximal values. Although some or all of the species were lacking in many of the water bottle samples, most of them were recorded in net samples throughout the year.

During a number of investigations larger standing crops of phytoplankton organisms have been recorded in coastal areas than in oceanic areas. As is seen from the preceding pages this is especially true for ceratia which are much more numerous along the Scandinavian coasts than they are in the open ocean, in certain localities the difference being as much as 10^5. The factors responsible for this are several and complicated. For the mass occurrence of ceratia it seems justifiable to assume that lower salinity and higher temperatures in the area discussed are important. With the above in mind, the following conclusions are proposed:

1. GRAN's "Tripos-Region" (1902) is a biogeographical area limited by summer temperature and salinity borders.

2. The localities for mass occurrences of ceratia in the Oslofjord and south of the Danish islands are areas which in summer and autumn offer nearly optimal temperature and salinity conditions for growth.

3. The low salinity in the Baltic forms a distribution border for ceratia which varies for the species according to their tolerance to brackish water.

The importance of light is not easily determined from investigation in natural surroundings since simultaneous observations of light intensity and phytoplankton populations are nearly completely lacking. Another confusing factor is that in the areas discussed fluctuations of light intensity during the year

parallel the fluctuations of salinity and temperature to a certain degree. Division rates of the ceratia in question, however, are high enough at low light intensities to provide for a considerable growth even in late autumn.

According to observations from the sea *C. lineatum* is commonly represented in too low quantities for any marked correlation with experimental data. Despite its ability to thrive at low salinity and high temperature and light intensity at seemingly favourable nutrient conditions, it is never recorded in such immense numbers as are *C. tripos*, *C. fusus* and *C. furca*.

V. Summary

1. The organisms used in the experiments were *Ceratium tripos*, *C. fusus*, *C. furca* and *C. lineatum*.

2. The methods for cultivation are described in detail, the more important points of which are: Ceratia must be handled more carefully than other dinoflagellates owing to the size and shape. They should not be subjected to temperature or salinity shocks during isolation and other transfers.

3. The growth rate in unicellular and multicellular cultures was investigated together with the effect of culture density upon growth rate. The division rate in cultures was found to lie within the ranges found in natural populations, and the *Ceratium* cultures behaved more or less like other cultures with exponential growth phases.

4. The optimal salinity for the *Ceratium* species in question was found to lie at 15–25‰ with a fairly large tolerance range.

5. The optimal temperature lay at 15–20°C, but the tolerance for lower temperatures varied considerably with the species.

6. The optimal light intensity was found to lie at approximately 5000 Lux for *C. tripos* and *C. lineatum*, and 2500 Lux for *C. fusus* and *C. furca*, intermediate values not investigated. Owing to the unchecked effects of phototaxis, the values given may be more or less erroneous.

7. Experiments with oceanic and neritic water for culture solutions revealed that ocean water is not satisfactory for preparation of Schreiber's solution. This medium, however, was considerably improved by an addition of soil extract. This was also true with neritic water. In aged water from the Oslofjord the addition of P + N did not improve the solution, indicating that this water contains enough of these elements for phytoplankton growth.

8. An investigation on the variation of the length of cells grown under different culture conditions showed that the average cell lengths of *C. fusus*, *C. furca* and *C. lineatum* were shorter in cultures than in natural populations, decreasing with increasing temperature and culture density. The cell lengths were also shorter in neritic than in oceanic water.

9. The variation of cell forms in clone cultures was demonstrated, with examples given indicating that abnormal nuclear fission is characteristic of aberrant cell forms.

10. An observation of bioluminescence in *Ceratium fusus* cultures was reported.

11. A comparison of temperature and salinity experiments and observations from Northern European coastal waters was undertaken. The results support the assumption that: 1) in the Baltic low salinity restricts the distribution of the *Ceratium* species in question; 2) the mass occurrence of ceratia in the Oslofjord in summer partly may be ascribed to the favourable temperature and salinity conditions in this locality; 3) the Norwegian coastal waters, Gran's "Triposregion", is a biographical area which offers ceratia better growth conditions than the colder and saltier oceanic water to the west.

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Table I. *Ceratium tripos*.

Daylight. Room temperature. 4–21 July 1949. "Erdschreiber".

Parallels	Initial number of cells	Number of cells after n days								
		3	4	5	7	8	10	12	14	17
I	10	22	30	34	48	51	70	93	112	136
II	10	17	26	34	51	56	68	115	155	203
III	10	16	22	24	33	33	46	57	77	95
IV	10	16	30	31	53	60	77	112	134	159
V	10	?	24	26	52	55	82	116	145	190
VI	10	?	14	18	29	23	54	54	61	69
Sum	60	71	146	167	266	278	397	547	684	851
One cell has multiplied to		1.78	2.43	2.78	4.43	4.63	6.62	9.12	11.40	14.18
Number of divisions. . . .		0.83	1.28	1.48	2.15	2.21	2.73	3.19	3.51	3.81
Number of divisions per 24 hours.		0.28	0.32	0.30	0.31	0.28	0.27	0.27	0.25	0.22

Counted in the culture vessels during the experiment.

Table II. *Ceratium tripos.*

"Electric sun". Room temperature. "Erdschreiber".

7–12 Aug. 1948. 300 W bulb.			14 Aug.–13 Sept. 1948. 200 W bulb.		
Parallels	Initial number of cells	End result	Parallels	Initial number of cells	End result
I	10	21	I	5	28
II	10	15	II	5	33
III	10	16	III	5	52
IV	10	12	IV	5	14
V	10	19	V	5	77
Sum	50	83		25	204
One cell has multiplied to		1.67	–	–	8.04
Number of divisions.....		0.74	–	–	3.09
Number of divisions per 24 hours.....		0.15	–	–	0.11

Table III. *Ceratium tripos.*

Daylight. Room temperature. 25 July–12 August 1950. "Erdschreiber".

Parallels	Initial number of cells	Number of cells after n days				
		3	7	10	15	18
I	1	2	4	7	12	17
II	1	1	5	8	14	16
III	1	3	6	17	30	40
IV	1	2	3	3	6	9
V	1	2	4	10	14	14
Sum	5	10	22	45	76	96
One cell has multiplied to		2.00	4.40	9.00	15.20	19.20
Number of divisions.....		1.00	2.14	3.17	3.93	4.26
Number of divisions per 24 hours.....		0.33	0.31	0.32	0.26	0.24

Table IV. *Ceratium fusus.*

Daylight. Room temperature. "Erdschreiber".

Parallels	22 July–12 Aug. 1948		14 Aug.–10 Sep. 1948	
	Average initial number of cells	End result	Initial number of cells	End result
I	235	3400	5	173
II	235	2500	5	130
III	235	2350	5	182
IV	235	1460	5	177
V	(235)	(110)	5	85
Sum	940	10610	25	747
One cell has multiplied to		11.24	–	29.88
Number of divisions		3.50	–	4.90
Number of divisions per 24 hours		0.17	–	0.18

Table V. *Ceratium fusus.*

300 W bulb. Room temperature. "Erdschreiber". 3–17 March 1949.

Parallels	Initial number of cells	End result	Initial number of cells	End result	Initial number of cells	End result
I	1	25	10	33	50	180
II	1	79	10	42	50	126
III	(1)	–	10	61	50	255
IV	1	45	10	11	50	114
V	(1)	–	10	50	50	74
Sum	3	149	50	197	250	749
One cell has multiplied to		49.67	–	3.94	–	3.00
Number of divisions		5.63	–	1.98	–	1.59
Number of divisions per 24 hours		0.40	–	0.14	–	0.11

Table VI. *Ceratium furca.*

Daylight. Room temperature. "Erdschreiber". July–August 1949.

Growth period	Initial number of cells	End result	One cell has multiplied to	Number of divisions	Number of divisions per 24 hours
14 days	8	523	65.38	6.03	0.43
21 –	10	9750	975.00	9.93	0.47
27 –	5	4350	869.80	9.77	0.36

Table VII. *Ceratium furca.*

Daylight. Room temperature. "Erdschreiber". 24 July–9 August 1949.

Parallels	Initial number of cells	Number of cells after n days	
		7	13
I	1	11	52
II	1	16	47
Sum	2	27	99
One cell has multiplied to		13.50	49.50
Number of divisions.....		3.76	5.63
Number of divisions per 24 hours..		0.54	0.43

Table VIII. *Ceratium furca.*

Fluorescent tubes, 900 Lux. 2 ml "Erdschreiber" in staining vessels. 25 Jan.–12 Feb. 1952.

Parallels	Initial number of cells	Number of cells after n days											
		1	3	4	6	7	8	10	11	12	13	15	18
I	1	1	3	4	8	8	13	26	33	44	62	77	147
II	1	1	2	4	7	7	11	18	18	27	27	44	69
III	1	1	2	4	8	12	16	29	30	41	49	49	79
IV	1	1	4	4	4	9	12	17	26	41	41	53	102
Sum	4	4	11	16	27	36	52	90	107	153	179	223	397
One cell has multiplied to.....		1	2.75	4.00	6.75	9.00	13.00	22.50	26.75	38.25	44.75	55.75	99.25
Number of divisions.		0	1.46	2.00	2.75	3.18	3.70	4.50	4.74	5.26	5.48	5.80	6.63
Number of divisions per 24 hours.....			0.49	0.50	0.46	0.45	0.46	0.45	0.43	0.44	0.42	0.39	0.37

Table IX. *Ceratium longipes.*

Daylight. Room temperature. "Erdschreiber". 9–30 May 1951.

Parallels	Initial number of cells	Number of cells after n days		
		7	14	21
I	1	4	19	35
II	1	2	23	62
III	1	2	24	69
IV	1	2	22	62
V	1	3	22	60
VI	1	1	12	22
VII	1	2	12	26
VIII	1	1	6	25
IX	1	2	19	51
X	1	1	9	27
Sum	10	20	168	439
One cell has multiplied to		2.00	16.80	43.90
Number of divisions.....		1.00	4.07	5.46
Number of divisions per 24 hours.....		0.14	0.29	0.26

Table X. *Ceratium tripos.*

Fluorescent tubes, 1500 Lux. Room temperature. 3 Sep.–29 Oct. 1953.

Ml of fluid	Initial number of cells per ml	Number of cells per ml (a) and division rate per 24 hours (b) after n days							
		14		28		42		56	
		a	b	a	b	a	b	a	b
30	32	132	0.15	535	0.15	2024	0.14	1700	0.10
60	16	69	0.15	208	0.13	846	0.14	1550	0.12
120	8	18	0.08	58	0.10	114	0.09	167	0.08
240	4	7	0.06	45	0.12	57	0.09	59	0.07
Ml counted ...	30	10		10		1		1	

Table XI. *Ceratium furca.*

Fluorescent tubes, 1500 Lux. Room temperature. 22 Jan.–19 Feb. 1953.

Ml of fluid	Initial number of cells per ml	Number of cells per ml (a) and division rate per 24 hours (b) after n days							
		7		14		21		28	
		a	b	a	b	a	b	a	b
30	28	48	0.11	146	0.17	641	0.22	1715	0.21
60	14	32	0.17	99	0.20	283	0.21	870	0.21
120	7	18	0.20	57	0.22	208	0.24	838	0.25
240	3.5	8	0.17	41	0.26	170	0.27	693	0.27
Ml counted ...	30	10		10		1		1	

Table XII. *Ceratium lineatum.*

Fluorescent tubes, 1500 Lux. Room temperature. 18 Nov.–12 Dec. 1953.

Ml of fluid	Initial number of cells per ml	Number of cells per ml (a) and division rate per 24 hours (b) after n days							
		7		14		21		28	
		a	*b*	*a*	*b*	*a*	*b*	*a*	*b*
30	132	443	0.25	1380	0.24	5480	0.26	8120	0.21
60	66	191	0.23	790	0.26	3700	0.28	4700	0.22
120	33	90	0.21	271	0.21	1815	0.28	5110	0.27
240	17	47	0.21	174	0.24	768	0.26	635	0.31
Ml counted ...	10	2		1		0.1		0.1	

Table XIII. *Ceratium tripos.*

Daylight. Room temperature. "Erdschreiber". 9–30 Sep. 1949.

Parallels	Initial number of cells	‰ S						
		10	15	20	25	30	35	40
I	10	36	351	657	87	108	77	143
II	10	37	309	577	267	111	29	30
III	10	–	346	529	120	24	7	46
IV	10	73	342	253	291	–	70	66
V	10	58	265	1380	411	168	205	58
Sum	50	204	1713	3396	1176	411	388	343
One cell has multiplied to		5.01	34.26	67.95	23.52	10.85	7.76	6.86
Number of divisions.....		2.33	5.10	6.09	4.56	3.44	2.96	2.78
Number of divisions per 24 hours		0.11	0.24	0.29	0.22	0.16	0.14	0.13
Counted.....					Total			

Table XIV. *Ceratium fusus.*

Daylight. Room temperature. "Erdschreiber". 29 July–17 Aug. 1947.

Parallels	Initial number of cells	‰ S						
		10	15	20	25	30	35	40
I	25	–	307	1009	2340	484	420	75
II	25	–	341	2950	2230	455	147	82
III	25	–	92	2980	1082	785	81	262
IV	25	–	226	2000	2120	974	122	47
V	25	–	352	2270	927	671	73	293
Sum	125	–	1318	11209	8699	3369	843	705
One cell has multiplied to		–	10.54	89.70	69.60	26.95	6.76	5.64
Number of divisions.....		–	3.40	6.49	6.12	4.75	2.76	2.50
Number of divisions per 24 hours		–	0.18	0.34	0.32	0.25	0.15	0.13
Counted.....					Total			

Table XV. *Ceratium furca.*

Daylight. Room temperature. "Erdschreiber". 27 Aug.–16 Sep. 1949.

Parallels	Initial number of cells	‰ S						
		10	15	20	25	30	35	40
I	10	–	425	1497	1473	1083	357	112
II	10	–	410	1890	2202	1161	213	41
III	10	–	261	1644	2031	1260	291	106
IV	10	–	531	2664	1518	1356	897	128
V	10	–	593	1173	1479	1425	501	156
Sum	50	–	2220	8850	8721	6285	2259	543
One cell has multiplied to		–	44.40	177.00	174.40	125.70	45.18	11.00
Number of divisions.....		–	5.47	7.47	7.45	6.97	5.50	3.46
Number of divisions per 24 hours		–	0.26	0.36	0.36	0.33	0.26	0.17
Counted.....		Total			10 ml		Total	

Table XVI. *Ceratium lineatum.*

Fluorescent tubes. 800 Lux. Room temperature. "Erdschreiber". 3–16 March 1952.

Parallels	Initial number of cells	‰ S						
		10	15	20	25	30	35	40
I	10	–	320	571	282	20	9	–
II	10	–	530	385	174	24	26	–
III	10	–	312	807	251	129	19	–
IV	10	–	414	528	268	45	25	–
V	10	–	285	254	267	37	29	–
Sum	50	–	1861	2446	1242	255	108	–
One cell has multiplied to		–	37.22	48.92	24.84	5.10	2.16	–
Number of divisions.....		–	5.22	5.61	4.63	2.33	1.11	–
Number of divisions per 24 hours		–	0.40	0.43	0.36	0.18	0.08	–
Counted.....					Total			

Table XVII. *Ceratium tripos.*

Flourescent tubes, 800 Lux. Thermostate. "Erdschreiber". 3–21 April 1954.

Parallels	Initial number of cells	°C				
		5	10	15	20	25
I	25	543	605	636	1176	276
II	25	516	504	501	1098	117
III	25	504	549	663	951	366
IV	25	531	603	609	531	354
V	25	477	492	717	768	426
Sum	125	2571	2751	3126	4524	1539
One cell has multiplied to		20.57	22.01	25.01	36.19	12.31
Number of divisions		4.36	4.46	4.64	5.18	3.62
Number of divisions per 24 hours		0.24	0.25	0.26	0.29	0.20
Counted				10 ml		

Table XVIII. *Ceratium fusus.*

Fluorescent tubes, 800 Lux. Thermostate. "Erdschreiber". 1–21 Dec. 1952.

Parallels	Initial number of cells	°C				
		5	10	15	20	25
I	25	–	262	1040	656	281
II	25	–	268	1622	816	108
III	25	–	234	1430	522	144
IV	25	–	248	1080	424	299
V	25	–	306	2680	276	230
Sum	125	–	1354	7852	2694	1062
One cell has multiplied to		–	10.83	62.82	21.55	8.50
Number of divisions		–	3.43	5.97	4.43	3.09
Number of divisions per 24 hours		–	0.17	0.30	0.22	0.15
Counted		Total		15 ml	Total	

Table XIX. *Ceratium furca.*

Fluorescent tubes, 800 Lux. Thermostate. "Erdschreiber". 15 May–10 June 1952.

Parallels	Initial number of cells	°C				
		5	10	15	20	25
I	10	–	49	1074	3252	234
II	10	–	48	888	4737	732
III	10	–	47	1383	5052	705
IV	10	–	38	1812	3821	516
V	10	–	56	2025	4836	552
Sum	50	–	238	7182	21708	2739
One cell has multiplied to		–	4.76	143.64	434.16	54.82
Number of divisions.....		–	2.25	7.17	8.76	5.78
Number of divisions per 24 hours.....		–	0.07	0.28	0.34	0.22
Counted.....		Total			10 ml	Total

Table XX. *Ceratium lineatum.*

Fluorescent tubes, 800 Lux. Thermostate. "Erdschreiber". 28 March–18 April 1952.

Parallels	Initial number of cells	°C				
		5	10	15	20	25
I	10	11	98	270	1632	305
II	10	22	134	281	1120	877
III	10	23	168	352	266	409
IV	10	17	119	57	676	905
V	10	18	82	242	3120	615
Sum	50	91	601	1202	7314	3111
One cell has multiplied to		1.82	12.02	24.04	146.28	62.22
Number of divisions.....		0.86	3.59	4.59	7.19	5.96
Number of divisions per 24 hours.....		0.04	0.17	0.22	0.34	0.28
Counted.....			Total		15 ml	Total

Table XXI. *Ceratium tripos.*

Daylight. Room temperature. "Erdschreiber". 8–20 Aug. 1949.

Parallels	Initial number of cells	Relative light intensity			
		1/4	1/2	3/4	1/1
I	10	45	168	185	236
II	10	58	105	236	172
III	10	165	133	177	181
IV	10	180	185	149	187
Sum	40	448	591	749	776
One cell has multiplied to		11.20	14.80	18.70	19.40
Number of divisions		3.49	3.89	4.22	4.28
Number of divisions per 24 hours		0.29	0.32	0.35	0.35
Counted		Total			

Table XXII. *Ceratium furca.*

Daylight. Room temperature. "Erdschreiber". 8–20 Aug. 1949.

Parallels	Initial number of cells	Relative light intensity			
		1/4	1/2	3/4	1/1
I	10	22	101	187	154
II	10	52	103	71	223
III	10	27	–	55	142
IV	10	39	60	118	178
Sum	40	140	264	431	697
One cell has multiplied to		3.50	8.80	10.78	17.42
Number of divisions		1.80	3.14	3.43	4.12
Number of divisions per 24 hours		0.15	0.26	0.29	0.34
Counted		Total			

Table XXIII. *Ceratium tripos.*

Fluorescent tubes. Room temperature. "Erdschreiber". 1–17 Sep. 1953.

Parallels	Initial number of cells	Lux				
		500	1000	2500	5000	10000
I	10	16	64	63	140	58
II	10	18	30	53	121	127
III	10	10	50	33	81	61
IV	10	10	13	47	102	139
V	10	16	28	151	166	58
Sum	50	70	185	347	610	443
One cell has multiplied to		1.40	3.70	6.94	12.20	8.86
Number of divisions.....		0.48	1.89	2.79	3.61	3.15
Number of divisions per 24 hours.....		0.03	0.12	0.17	0.23	0.20
Counted.....				Total		

Table XXIV. *Ceratium fusus.*

Fluorescent tubes. Room temperature. 50 ml "Erdschreiber" in 50 ml Erlenmeyer flasks. 18 May–2 June 1953.

Parallels	Initial number of cells	Lux				
		500	1000	2500	5000	10000
I	31	140	685	1420	500	470
II	31	220	715	1490	795	335
III	31	230	820	1130	570	455
IV	31	320	545	1380	505	560
Sum	124	910	2765	5420	2370	1820
One cell has multiplied to		7.34	22.30	43.71	19.11	14.68
Number of divisions.....		2.88	4.48	5.45	4.25	3.88
Number of divisions per 24 hours.....		0.19	0.30	0.36	0.28	0.26
Counted.....				10 ml		

Table XXV. *Ceratium furca.*

Fluorescent tubes. Room temperature. "Erdschreiber". 3–17 Nov. 1952.

Parallels	Initial number of cells per ml	Lux				
		500	1000	2500	5000	10000
I	32	38	139	1051	118	–
II	32	40	374	1156	155	–
III	32	59	443	1130	155	–
IV	32	99	283	1601	126	–
V	32	137	117	1516	119	–
Sum	160	373	1356	6454	673	–
One cell has multiplied to		2.33	8.48	40.34	4.21	–
Number of divisions.....		1.22	3.08	5.33	2.07	–
Number of divisions per 24 hours.....		0.09	0.22	0.38	0.15	–
Counted.....				1 ml		

Table XXVI. *Ceratium lineatum.*

Fluorescent tubes. Room temperature. "Erdschreiber". 17 Nov.–2 Dec. 1952.

Parallels	Initial number of cells per ml	Lux				
		500	1000	2500	5000	10000
I	70	988	2650	5400	7450	2280
II	70	1044	2570	5510	10210	–
III	70	954	2110	5420	7410	1258
IV	70	1092	2400	5930	10260	1214
V	70	963	1830	5350	10820	1490
Sum	350	5041	11560	27610	46155	6250
One cell has multiplied to		14.40	33.03	78.89	137.87	17.86
Number of divisions.....		3.85	5.05	6.30	7.03	4.16
Number of divisions per 24 hours.....		0.26	0.34	0.42	0.47	0.28
Counted.....		1 ml	1 ml of culture diluted 1:10			1 ml

Table XXVII. *Ceratium furca.*

Fluorescent tubes, 800 Lux. Sea water with soil extract and with varying concentrations of P + N. 9–23 June 1952.

Parallels	Initial number of cells	Concentration of P+N			
		0	0.1	1	10
I	10	476	515	538	15
II	10	569	426	475	42
III	10	354	291	561	76
IV	10	475	458	385	72
V	10	492	593	425	67
Sum	50	2366	2283	2384	272
One cell has multiplied to		47.32	45.66	47.68	5.44
Number of divisions		5.56	5.51	5.58	2.33
Number of divisions per 24 hours		0.40	0.39	0.40	0.17
Counted		Total			

Table XXVIII. *Ceratium furca.*

Fluorescent tubes, 800 Lux. Schreiber's solution with varying concentrations of soil extract. 9–23 June 1952.

Parallels	Initial number of cells	Concentration of soil extract			
		0	0.1	1	10
I	10	176	177	538	62
II	10	224	235	475	31
III	10	128	96	561	20
IV	10	110	144	385	29
V	10	116	191	425	40
Sum	50	754	843	2384	182
One cell has multiplied to		14.08	16.86	47.68	3.64
Number of divisions		3.82	4.08	5.58	1.86
Number of divisions per 24 hours		0.27	0.29	0.40	0.13
Counted		Total			

Table XXIX. *Ceratium furca.*

Fluorescent tubes, 800 Lux. Schreiber's solution with varying concentrations of soil extract. 10 Nov.–2 Dec. 1952.

Parallels	Initial number of cells	Neritic Schr. + ml/l soil extract					
		0	10	25	50	100	200
I	25	187	279	736	666	792	107
II	25	107	777	320	124	900	63
III	25	188	472	372	442	870	72
IV	25	158	670	367	992	1004	83
V	25	45	480	346	142	712	73
Sum	125	685	2670	2737	2366	4338	395
One cell has multiplied to		5.48	20.88	17.10	18.93	34.70	3.18
Number of divisions.....		2.45	4.38	4.10	4.24	5.12	1.67
Number of divisions per 24 hours..		0.11	0.20	0.19	0.19	0.23	0.08
Counted.....		Total					

Table XXX. *Ceratium furca.*

Fluorescent tubes, 800 Lux. Schreiber's solution with varying concentrations of soil extract. 10 Nov.–2 Dec. 1952.

Parallels	Initial number of cells	Oceanic Schr. + ml/l soil extract					
		0	10	25	50	100	200
I	25	–	159	486	233	536	25
II	25	–	598	224	846	786	44
III	25	–	574	248	114	670	69
IV	25	–	570	284	994	872	38
V	25	–	444	306	600	400	29
Sum	125	–	2375	1548	2787	3204	205
One cell has multiplied to		–	18.52	12.38	22.30	25.63	1.64
Number of divisions.....		–	4.27	3.63	4.48	4.68	0.71
Number of divisions per 24 hours..		–	0.19	0.17	0.20	0.21	0.03
Counted.....		Total					

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