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Total dissolved carbohydrates in an enclosure experiment with unialgal *Skeletonema costatum* culture

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ABSTRACT: In a southern Norwegian fjord (Rosfjord) during POSER a plastic enclosure (1 m ∅, 20 m depth) was filled with filtered fjord water enriched with inorganic nutrients and inoculated with a monoculture of *Skeletonema costatum*. The enclosure was exposed to fjord conditions from March 18 to April 5, 1979. Pre-filtration had removed ca. 80 % of the bacterial population in the fjord water. Following a short lag phase, bacteria grew exponentially. After consumption of nitrogen-containing organic substances, dissolved carbohydrates were the main energy source; however, bacterial numbers did not increase further, probably due to grazing by nanoflagellates. In spite of sub-optimal light and temperature conditions, *S. costatum* reached cell densities as high as $35 \cdot 10^6$ dm^{-3} at 3 m water depth, since competing algae and grazing zooplankton were missing in the enclosure. The diatoms grew exponentially, with a division rate of $\mu = 0.6$ d^{-1} and reached the stationary phase after 16 d, when inorganic nutrients were exhausted. During growth of *S. costatum* relatively high amounts of carbohydrates were released. Maximum concentrations followed the exponential phase, but highest release rates per cell – 2.0 pmoles glucose equivalents $cell^{-1}$ d^{-1} – were reached immediately after inoculation. Measurements at 3 h intervals at the end of the experiment showed that concentrations and therefore release activities varied abruptly within a few hours. Main release occurred from noon to midnight. Not all dissolved carbohydrates were immediately taken up by heterotrophic organisms, part of them could not be utilized, others only after adaptation or succession of bacteria. During the experiment, the proportion of the labile fraction to the total dissolved carbohydrates varied greatly.

INTRODUCTION

As is well known, healthy phytoplankters release considerable amounts of photosynthetic products during development (Lewin, 1956; Prager et al., 1959; Guillard and Hellebust, 1971; Ignatiades and Fogg, 1973; Aaronson, 1978; Sharp, 1978; Mague et al., 1980). Carbohydrates are one of the main components of the organic substances released (Degens, 1970; Handa, 1970; Hellebust, 1974; Bolze and Soeder, 1978). Possibly, they play an important role in aquatic food chains.

An objective of this work was a detailed investigation of the release of dissolved carbohydrates during phytoplankton development as a function of different growth phases. Because of horizontal advection, measurements in the open sea do not seem an appropriate means for such investigations. Enclosures containing unfiltered sea water do not provide much better conditions, since normally in such experiments many plankters develop simultaneously (Brockmann et al., 1977 b, 1979; Kattner et al., 1983). A bloom of one particular species is achieved more easily when sea water is filtered, enriched with inorganic nutrients and inoculated with a monoculture of phytoplankton. Such experiments were carried out in large plastic enclosures (tanks) during POSER in a south Norwegian fjord in 1979 (Brockmann et al., 1983). For inoculation, diatom species which dominated in the fjord during the experiment were precultured (Jahnke et al., 1983). This paper reports results of a tank experiment with *Skeletonema costatum*. Inorganic nutrients were added in relatively small quantities, equivalent to about maximum concentrations in the fjord. In this

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way, results for carbohydrate release were obtained which occurred under nearly natural conditions. Since monocultures in our tanks cannot be kept axenic, prefiltration of the water was carried out in such a way that natural bacteria and other microorganisms as nanoflagellates were not removed completely.

Many investigators have attempted to demonstrate stimulation of bacterial growth by algal extracellular products, of which carbohydrates comprise the substances of most interest (for review see Bell and Sakshaug, 1980); possible antibiotic effects have remained less studied (Sieburth, 1968). However, compared to grazing by protozoans, antibiosis is now considered to be of little importance for the removal of coliform bacteria in estuarine waters (Enzinger and Cooper, 1976). Generally, stimulation of bacterial numbers and production in the productive season in temperate waters is well documented (e.g. Hagström et al., 1979). Such stimulation is related to algal extracellular release during growth, as well as to substances released during senescence (Sieburth, 1979). Thus, an additional objective of this work was the investigation of the growth of heterotrophic bacteria during the developing diatom bloom.

METHODS

In the Rosfjord a plastic tank (1 m ∅, 20 m depth) was filled on March 16, 1979, with plate-filtered fjord water of 29.6 ‰ salinity and exposed from March 18 to April 5 (Brockmann et al., 1983). Plate filters (Type 2/1250) were obtained from Seitz (Bad Kreuznach, F.R.G.); they hat a pore size of approximately 0.2 µm. By enrichment of the water with inorganic nutrients the following concentrations were obtained: 11.2 µg at nitrate-N dm^{-3}, 0.8 µg at o-phosphate-P dm^{-3}, and 7.5 µg at silicate-Si dm^{-3}. Initial ammonia concentrations were approximately 1 µg at ammonia-N dm^{-3}. The water was inoculated with a monoculture of *Skeletonema costatum* on March 18 (Jahnke et al., 1983). On March 21, the tank was re-inoculated with 5 isolates of bacteria for ecological studies (Dahle and Laake, 1982).

Samples were taken at 0, 3, 10 and 15 m depth (Brockmann and Hentzschel, 1983; Brockmann et al., 1983). To count of colony forming units (CFU), triplicate 0.1 ml spread plates were prepared on a pepton trypton, yeast extract medium (0.5 g dm^{-3} of each in 1.5 % sea water agar; Dahle and Laake, 1982) and incubated at 10°C for 21 d. Samples of 10 ml were preserved with neutralized formaldehyde (2 % final concentration) until prepared for acridine orange total counts (AOTC) by epifluorescence microscopy (Hobbie et al., 1977). For diatom counting (Wild inverted microscope), samples were fixed with neutralized formalin. Particles were counted immediately after sampling with a Coulter Counter TA II in raw water samples.

For chemical analyses, filtration was performed under constant vacuum (0.5 at) using GF/C (Whatman) glass-fibre filters which are known to have a retention ability of 1.2 µm. Nutrient measurements were carried out immediately after sampling and filtration. Samples for carbohydrate determination were fixed with 3 ml dm^{-3} of a 3.5 % (w/w) $HgCl_2$-solution and stored at about 4 °C.

Nutrients were measured with a Technicon Auto-Analyzer II, using methods for sea-water determinations recommended by Technicon (Methods No. 154–71 W, 155–71 W, 158–71 W, 186–72 W). They correspond to standardized methods of the Baltic countries (Carlberg, 1972). Nitrate plus nitrite, ammonia, ortho-phosphate and silicate were measured simultaneously from the same sample, using synthetic sea water (Technicon 158–71 W) as wash water. During the entire experiment the same stock standard which was preserved with chloroform (1 cm^3 dm^{-3}) was used. Working standards were prepared fresh daily with synthetic sea water as diluent. A low sampling frequency of 10 to 30 samples h^{-1} and a relatively long wash time were used, since base-lines sometimes drifted strongly, in spite of use of low-drift-phototubes and low-drift-amplifiers in the colorimeters. Between different samples many working standards were measured for calibration. Since ammonia in the air interfered with measurements of this parameter, sample cups were closed with a very thin membrane of Parafilm (Serva) which could be penetrated by the sharpened sampler needle.

Total dissolved carbohydrates were also determined with a Technicon AutoAnalyzer II using the L-tryptophan/sulfuric acid method I of Eberlein and Hammer (1980). Concentrations of interfering nitrate were measured with the conventional AutoAnalyzer method. By running nitrate standards for additional calibration of the carbohydrate channel it was possible to determine the nitrate reading which had to be substracted from the total reading. Since glucose was used for calibration of the carbohydrate determinations, concentrations were measured as glucose equivalents (Glc Eq).

RESULTS

Due to several influxes of cold, less haline water into the fjord (Brockmann et al., 1981) temperatures in the upper water layer and in the tank reached only 1 to 3 °C during the entire tank experiment; the deeper water in the fjord was warmer (2 to 6 °C) (Brockmann et al., 1981).

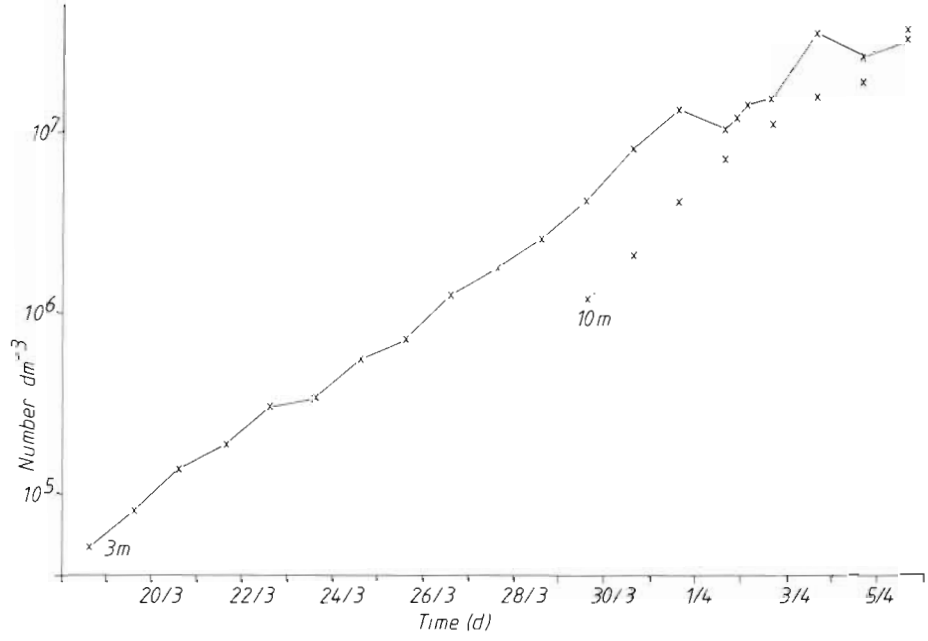

Fig. 1. *Skeletonema costatum*. Development of cell numbers at different depths in the enclosure (semi-logarithmic representation)

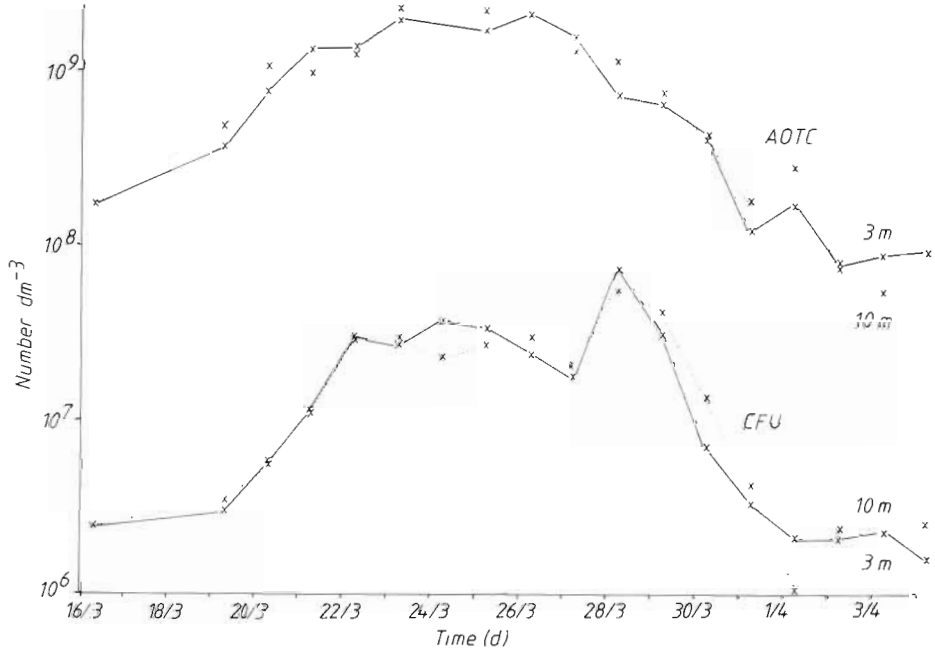

Fig. 2. Acridine orange total counts (AOTC) of bacteria and colony forming units (CFU) at different depths in the *Skeletonema costatum* tank (semi-logarithmic representation)

Growth of *Skeletonema costatum*

After adding *Skeletonema costatum* to the filtered and enclosed water on March 18, 1979 exponential growth started immediately (Fig. 1); initial density was 50,000 cells dm^{-3}. Daily cell division rate (μ) was as low as 0.6 and remained quite constant with the exception of the drop on April 1 to 2 at 3 m. Because of the low division rate, the exponential growth phase at 3 m lasted 16 d; the stationary phase was reached on April 3, 1979.

Diatom development at 10 m paralleled that at 3 m, but was delayed by 2 d. The stationary phase was reached at 10 m at the end of the experiment on April 5. Both, at 3 and 10 m, division rates were equally high after March 29, except for the drop on April 1 to 2.

Development of heterotrophic microorganisms

A comparison of acridine orange total counts (AOTC) of fjord and enclosure samples revealed that the pre-filtration removed ca. 80 % of the bacterial population. After 3 d (possibly including a 1 to 2 d lag phase) the bacteria in the enclosure grew exponentially for 4 d until March 23 (Fig. 2). The 5 strains used

for re-inoculation of the tank on March 21 comprised initially no more than 10 % of AOTC which may be considered insignificant in this context. The maximum specific growth rate for the AOTC population was μ = 0.62 d^{-1}; for colony forming units (CFU) of organotrophic bacteria it was μ = 1.4 d^{-1}. On March 22 to 23 a steady state developed; it lasted for about 6 d and was terminated by a rapid decline in both the AOTC and the CFU population to ca. 5 % of the maximum level. At both sampling depths (3 and 10 m) bacteria developed in a similar way (Fig. 2); temperatures also showed nearly no differences at these depths (Brockmann et al., 1981).

Coulter Counter measurements revealed that, in addition to bacteria and inoculated diatoms, particles with a size of 1.5 to 3 µm had increased in number. Distinction was possible because of different sizes and development of these particles from bacteria and diatoms. Increase of 1.5 to 3 µm particles was parallel at all 4 sampling depths; stratification could not be detected (Fig. 3). From March 24 to 29, a large increase was observed, interrupted by a decrease on March 25. On March 29, maximum values of about $25 \cdot 10^6$ particles dm^{-3} were measured. The period of fast growth of the 1.5 to 3 µm fraction coincided with the period of continuously high bacterial numbers. Nearly simultaneous with the beginning of decreasing bacterial numbers on March 30, a distinct decrease in the 1.5 to 3 µm particle fraction was observed. This fraction increased again slightly at the end of the experiment on April 4.

Consumption of inorganic nutrients

Except for ammonia, decreasing rates of nutrients were considered as uptake rates, since uptake proces-

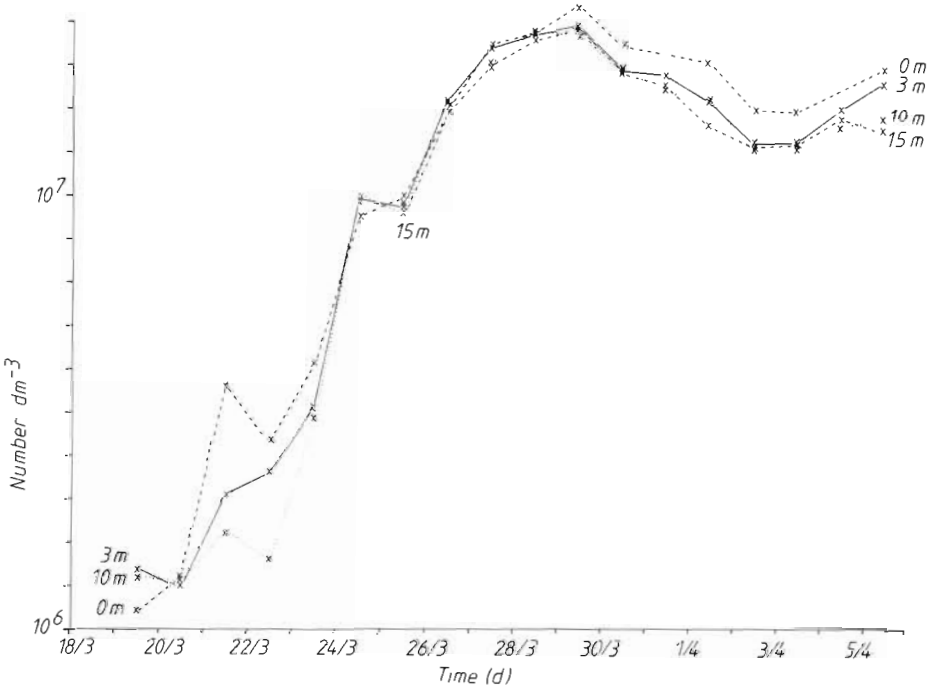

Fig. 3. Coulter Counter measurements of 1.5 to 3 µm particles at different depths in the *Skeletonema* tank (semi-logarithmic representation)

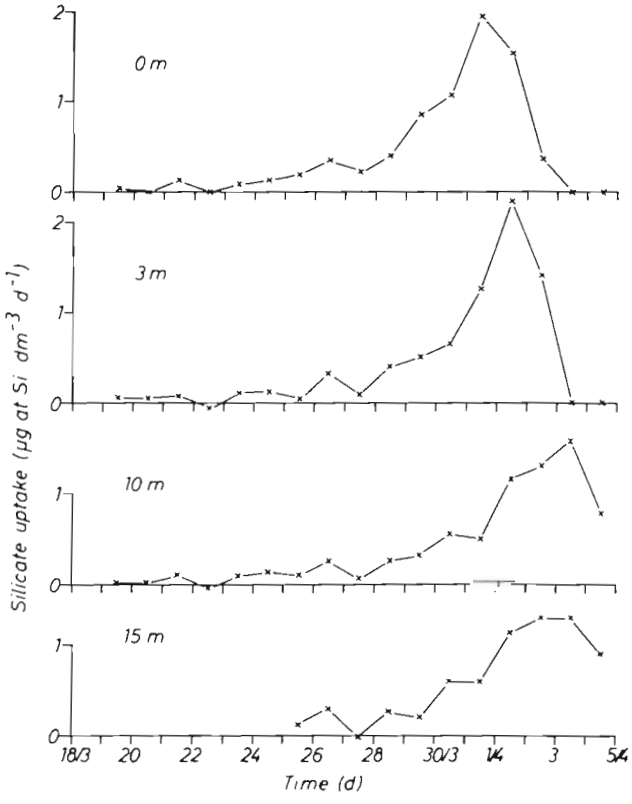

Fig. 4. Uptake of silicate at different depths in the *Skeletonema* tank; daily means

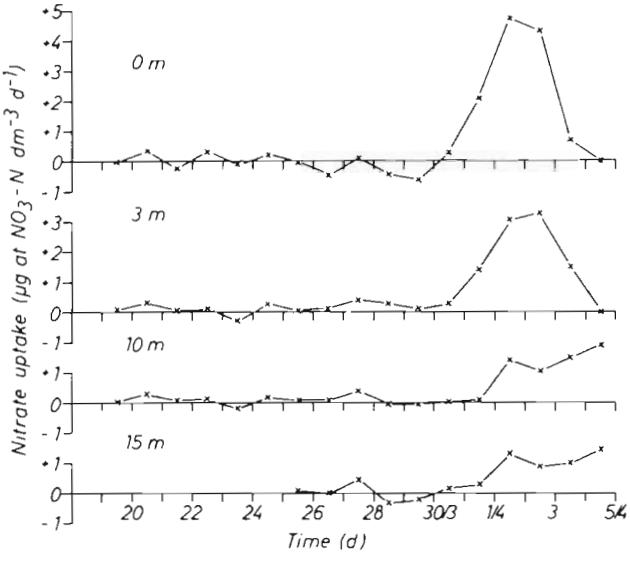

Fig. 5. Uptake of nitrate plus nitrite at different depths in the *Skeletonema* tank; daily means

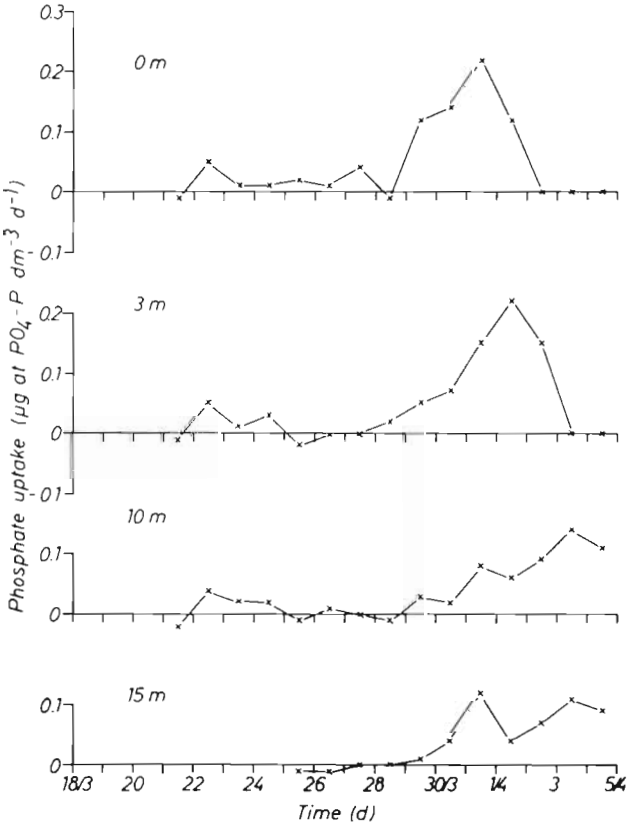

Fig. 6. Uptake of ortho-phosphate at different depths in the *Skeletonema* tank; daily means

ses were dominating, especially in the case of silicate and nitrate. The daily uptake in the enclosure is shown for 4 different sampling depths in Fig. 4 to 6. At the beginning of the experiment the water was enriched with nutrients to nearly the level of winter concentrations in this area. Uptake of silicate and phosphate was not evident at the surface until March 29; nitrate uptake started 2 d later. Strong nutrient uptake began later at lower sampling depths.

The uptake of the 3 inorganic nutrients showed a clear maximum at the surface and at 3 m. Maxima were reached first at the surface, later at increasing depth. Maxima of phosphate uptake coincided with those of silicate uptake; nitrate-uptake maxima were reached 1 d later.

Phosphate, nitrate and silicate were depleted early at 0 and 3 m depths, whereas at 10 and 15 m nutrients

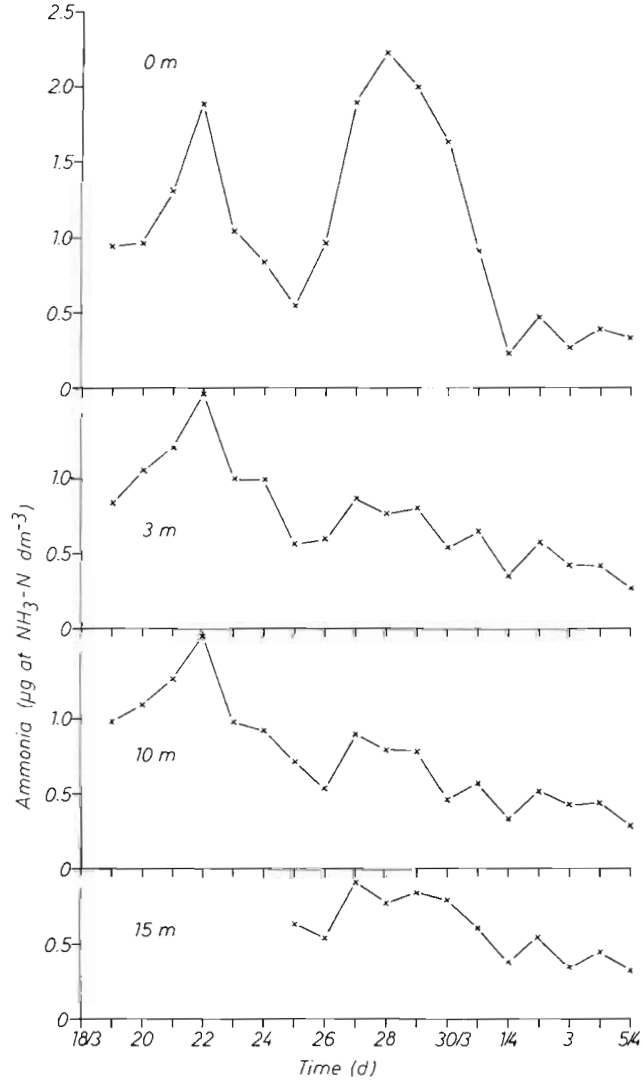

Fig. 7. Daily means of ammonia concentrations at different depths in the *Skeletonema* tank

were still available at the end of the experiment. At the surface, phosphate was exhausted by April 2, while silicate and nitrate were fully consumed 1 and 2 d

later, respectively. Nutrient depletion was delayed with increasing sample depth.

Since ammonia exhibited high concentration increases repeatedly, it was apparently not only taken up, but also released in relatively large amounts (Fig. 7). At all depths concentration maxima occurred near March 22 and March 28. The second maximum was much higher and more distinct at the surface than in the deeper water.

Concentrations of total dissolved carbohydrates

At the beginning of the experiment high concentrations of total dissolved carbohydrates (Fig. 8) were found in the 0.2 µm plate-filtered and enclosed water after sampling and filtration through glass-fibre filters (GF/C-Whatman, 1.2 µm retention ability). The same high concentrations of about 2.3 µmoles Glc Eq dm^{-3} were also measured in parallel samples additionally filtered through 0.1 µm membrane filters (Sartorius SM 11309). Carbohydrate concentrations remained high for 6 d, but decreased rapidly on March 23 and 24. The decrease occurred at the same time and to the same large amount at all depths (Fig. 9). After the rapid decrease, except for small fluctuations, carbohydrate concentrations remained in the low range of about 1.3 µmoles Glc Eq dm^{-3} until April 2 (Fig. 8). From March 18 to April 1 measurements of parallel samples, filtered through 0.1 µm filters as above, yielded similar concentrations as samples filtered only through 1.2 µm filters.

After April 2, extremely high carbohydrate concentrations were detected in single samples. Minimum values only increased relatively little from 1.3 to 1.8 µmoles Glc Eq dm^{-3} during this time. Concentration maxima on April 4 were much lower than those on April 3 and 5.

A detailed study of the carbohydrate concentrations at 3 m for the first 6 d of the experiment (Fig. 8) shows that, for the relatively small fluctuations during this time, a daily cycle can be detected. The highest concentrations of 2.3 to 2.5 µmoles Glc Eq dm^{-3} appeared at about noon, while at night a decrease to a value of about 2.2 µmoles Glc Eq dm^{-3} took place. A daily cycle was calculated by averaging decrease and increase rates at 6 h intervals over the first 6 d (Fig. 10). Due to a reduced sampling frequency at other depths the daily cycle of carbohydrate release could only be demonstrated at the depth of 3 m. The violent fluctuations in April (Fig. 8) did not run on the same, but on a similar cycle as those during the first 6 d of the experiment.

Whereas mean carbohydrate concentrations at all depths ran nearly parallel until April 1, this was not the case for the period from April 2 to 5 (Fig. 11). At 15 m no significant carbohydrate maxima were observed, while at 10 m they were highest. Also the peak patterns showed clear differences at different depths. Although carbohydrates were measured every 3 h at the end of the experiment, most peaks, especially at 10 m, are represented by only 1 measurement. A common feature of all sampling depths was that also during this period the highest carbohydrate concentrations appeared mainly during daylight, especially in the afternoon.

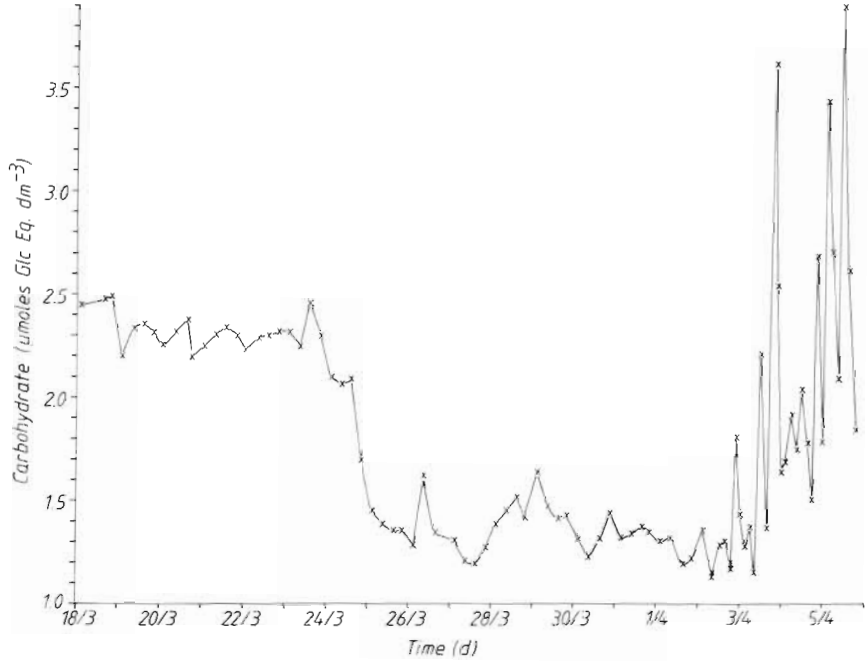

Fig. 8. Concentrations of total dissolved carbohydrates (measured as glucose equivalents) at 3 m in the *Skeletonema* tank

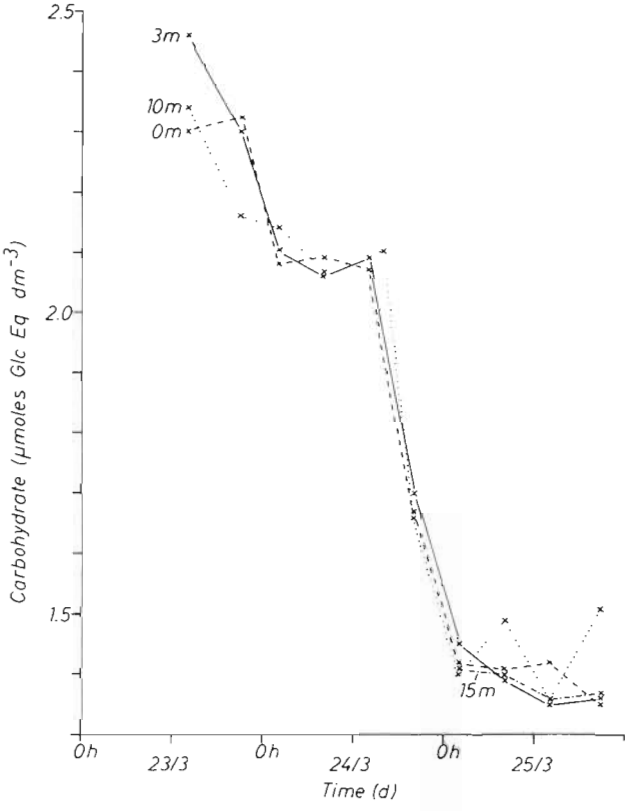

Fig. 9. Concentrations of total dissolved carbohydrates during period of high decrease, as in Fig. 8 but represented for different depths

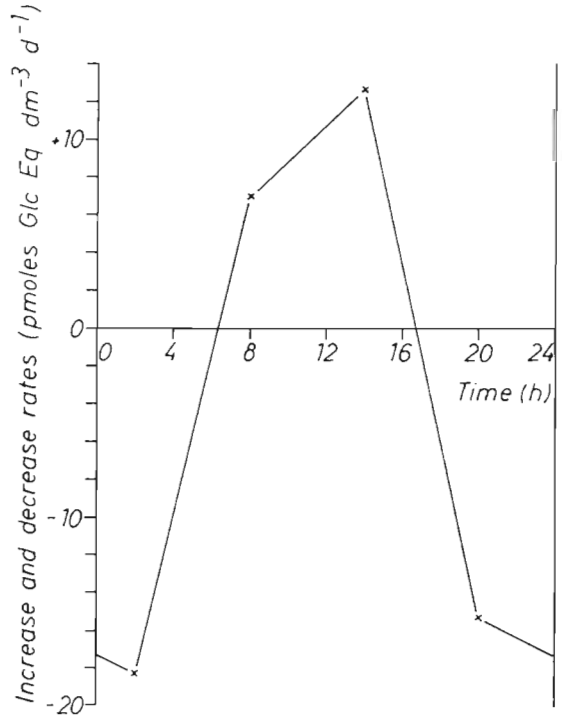

Fig. 10. Daily cycle of decrease and increase rates of total dissolved carbohydrates at 3 m in the *Skeletonema* tank, averaged data from March 19 to 23

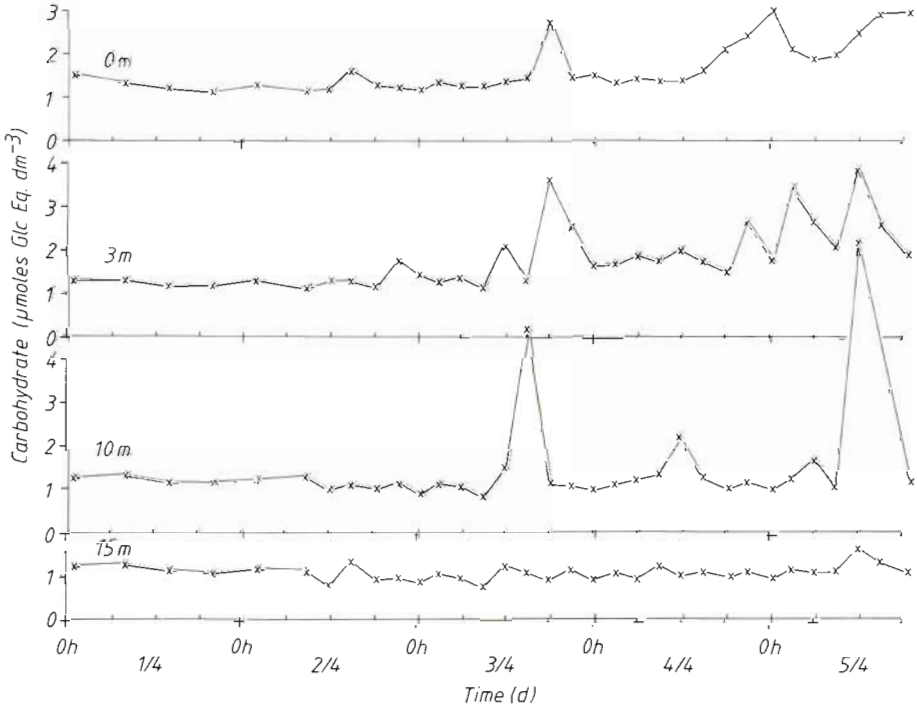

Fig. 11. Concentrations of total dissolved carbohydrates during period of maximum cell numbers of *Skeletonema costatum*, as in Fig. 8 but represented for different depths

DISCUSSION

Biological development in the enclosure

Growth of *Skeletonema costatum*

Due to experimental conditions, the inoculated diatom *Skeletonema costatum* was not the only organism in the enclosure and grew slowly. Its low division rate of $\mu = 0.6\ d^{-1}$ was caused to some extent by the low temperatures (1 to 3 °C); in the 1973 enclosure experiments with the same diatom, $\mu = 2.1$ to $2.4\ d^{-1}$ was found at 15.4 to 17.3 °C (Brockmann et al., 1977a). These results are in good accordance with those obtained in laboratory cultures (Jørgensen, 1968; Eppley, 1972) indicating that laboratory investigations on variations in growth rate with temperature can be useful guides for field work.

Field observations revealed that *Thalassiosira nordenskioeldii* is usually the dominant early spring diatom at 2 to 3 °C. It is replaced by other species, such as *Skeletonema costatum*, when water temperatures rise (Guillard and Kilham, 1977). Some weeks before starting our culture experiment with *S. costatum*, relatively high numbers of this diatom were found in the Rosfjord at 5 to 6 °C. After March 8, *S. costatum* could scarcely be detected in fjord samples due to low light intensities and later because of decreasing water temperatures (Kattner et al., 1983). Nevertheless this diatom grew exponentially in our culture tank.

Taking Eppley's formula (Eppley, 1977) for computation of maximal growth rates, *Skeletonema costatum* should reach $\mu = 1.0\ d^{-1}$ at 1 to 3 °C, provided that other environmental factors were optimal. However, only 0.6 divisions d^{-1} were found. Concentrations of inorganic nutrients should be considered satisfactory due to fertilization at the beginning of the experiment. But light intensities were perhaps not high enough for optimal development of *S. costatum* due to the early season and to shading influences. This can be deduced from the delay of diatom development at 10 m (Fig. 1) and by comparison of nutrient uptake at different depths (Fig. 4 to 6): Nitrate, phosphate and silicate uptake was delayed with increasing depth. Comparison of the mean light intensity of 15 n Einstein $cm^{-2}\ s^{-1}$, measured at 5 m depth during the experiment (Brockmann et al., 1982), with saturating light intensities of 13 to 32 n Einstein $cm^{-2}\ s^{-1}$, determined in laboratory cultures of *S. costatum* at relatively high temperatures (Eppley, 1977), does not reveal whether light limitation was the decisive factor or not.

In spite of sub-optimal conditions, *Skeletonema costatum* reached high cell densities ($35 \cdot 10^6\ dm^{-3}$) in the enclosure, obviously because competing algae and grazing zooplankters were absent. Moreover, vertical water exchange was limited in the tank. The drop of cell numbers on April 1 to 2 at 3 m (Fig. 1) cannot be explained.

Succession of organotrophic microorganisms

Bacteria were limited in their development earlier than diatoms (Fig. 2). Maximum increase rates of AOTC and CFU were found from March 19 to 22, coinciding with increased ammonia at all sampling depths (Fig. 7). This suggests decomposition processes by heterotrophic bacteria (Sorokin, 1978) and remineralization of proteins, amino acids and other nitrogen-containing substances assumed to have resulted in high concentrations from an algal bloom in the fjord before the water for the tank experiment was enclosed (see also p. 53). Moreover, an amino-acid containing pre-culture medium had been added to the tank by re-inoculation with bacteria isolates on March 21. The decrease in ammonia concentrations from March 22 to 25 (Fig. 7) indicates that utilizable nitrogen-containing substances ran low due to consumption by bacteria.

In 1973 tank experiments with *Skeletonema costatum*, a rapid reduction in heterotrophic bacteria (CFU) was observed when the algal population reached 10^6 cells dm^{-3}. This was attributed to effects of bactericidic substances excreted by the diatoms (Brockmann et al., 1977a). In the present experiment, however, an inhibitory effect of diatoms on bacteria is considered to be of little importance for the interpretation of the biological development because of the low division rate of *S. costatum*. Moreover, both the total and the heterotrophic population of bacteria developed in very similarly at both depths sampled, while the growth of *S. costatum* was delayed 2 d at 10 m compared to 3 m. No selective effect on the removal of any of the 5 strains counted by specific immunofluorescent staining was detected (Dahle and Laake, 1982), such as would be expected if bacteriolytic agents had been active.

A bacteriostatic effect cannot explain the rapid cell reduction observed in AOTC, since this method includes dead but morphologically intact cells, as well as dormant ones. A depletion of energy sources – e.g. available carbohydrates – although certainly governing bacterial growth rates and physiological state, cannot explain the rapid disappearance observed either. Bacterial aggregates were not frequently found in AOTC, and since there was no time lag in the disappearance of bacteria at 10 m compared to 3 m, this reduction cannot be due to sedimentation.

Most likely, the observed effect of decreasing bacteria numbers was due to overgrazing by phagotrophic nanoflagellates, which in recent years have been ascribed the role as dominant grazers on bacteria in

the sea (Seki, 1965; Lighthart, 1969; Sieburth et al., 1978; Haas and Webb, 1979; King et al., 1980). Other potential grazers on bacteria were not present in AOTC or phytoplankton samples. Unfortunately we did not examine nanoflagellate components microscopically in samples from this tank, but unidentified nanoflagellates were commonly present in the surrounding sea water at the time of this experiment (Brockmann et al., 1981); we have no other sound explanations for the rapid increase in the 1.5 to 3.0 µm fraction of particles observed after March 23 (Fig. 3). Some families of nanoflagellates (Bodonidae, Amphinomadidae, Monadidae) have cell diameters within this range, and have been demonstrated to feed voraciously on marine bacteria in culture (Haas and Webb, 1979; Laake and Holm-Hansen, unpubl.).

If correct, our observations and assumptions would represent the first published evidence for actual grazing of bacteria by nanoflagellates in a planktonic system under natural conditions. On the basis of this interpretation, the flagellates overgrazed their food resource and reduced it rapidly to a bacterial density (10^8 cells dm^{-3}) where no net energy could be obtained, since bacterial production probably was rate-limited by the availability of energy sources during the steady-state phase, as shown later for total carbohydrates. The steady-state phase of bacteria (Fig. 2) must be considered a dynamic equilibrium between rapid bacterial cell production, based on dissolved carbohydrates and other energy sources, and rapid cell removal by grazing.

The bacteria observed by epifluorescence microscopy were primarily small planktobacteria (Sieburth, 1979) with cell diameters from 0.4 to 0.6 µm, assumed to be most active in uptake of dissolved substrates (Hoppe, 1976; Azam and Hodson, 1977). However, also larger bacteria with diameters in the range of 0.7 to 0.9 µm were present in significant numbers. These may be considered epibacteria (Sieburth, 1979) primarily associated with particulate substrates and dominating the CFU data. On March 28 CFU comprized ca. 10 % of AOTC by numbers. A mean cell volume of 0.1 μm^3 in the AOTC population and 0.3 μm^3 in the CFU population was estimated. Assuming the observed maximum specific growth rate of 0.62 d^{-1} for the whole period of March 20 to 27 and taking into consideration a steady-state AOTC population of $2.2 \cdot 10^9$ cells dm^{-3}, a maximum daily production potential of $1.4 \cdot 10^9$ cells dm^{-3} d^{-1} can be estimated. By assuming a bacterial biomass of $1.21 \cdot 10^{-13}$ g C μm^{-3} cell volume (Watson et al., 1977), this equals $17 \cdot 10^{-6}$ g C dm^{-3} d^{-1}, which may be considered a rough estimate of the bacterial production potential during the period March 20 to 27. On March 18 to 19 the potential production was in the order of 10^{-6} g C dm^{-3} d^{-1}. From these estimates a maximum total bacterial production of about $140 \cdot 10^{-6}$ g C dm^{-3} is derived for the period March 18 to 27.

Assuming a 30 % efficiency in conversion of carbohydrates to bacterial biomass (Bell and Sakshaug, 1980), a bacterial production of $47.5 \cdot 10^{-6}$ g C dm^{-3} may result from an uptake of 2.2 µmoles Glc Eq dm^{-3} (see below). By using ammonia release values in addition, the uptake of nitrogen-containing organic substances was estimated which were utilized by deaminating processes. Assuming a C:N ratio of 7:1 for the nitrogen-containing substances (Hentzschel, pers. comm.), the release of 0.8 µmoles ammonia dm^{-3} during the period March 19 to 22 indicates a bacterial production of $20.2 \cdot 10^{-6}$ g C dm^{-3}. Both estimates lead to a possible bacterial production of $67.7 \cdot 10^{-6}$ g C dm^{-3}, a value in the same range as that computed from bacterial counts.

The AOTC increase of $9.4 \cdot 10^8$ cells dm^{-3} (Fig. 2) during March 19 to 21 at 3 m, corresponds to a production of $12 \cdot 10^{-6}$ g C; 80 % of this can be attributed to the uptake of nitrogen-containing substances, indicated by a release of 0.37 µmoles ammonia dm^{-3}, whereas 70 % of the supposed bacterial production from March 23 to 25 may be due to the uptake of dissolved carbohydrates amounting 1.1 µmoles Glc Eq dm^{-3} (see below).

Two to 3 d after the beginning of the increase in the 1.5 to 3 µm particle numbers (Fig. 3), ammonia concentrations at all depths began a second increase (Fig. 7). The very high ammonia concentrations at the surface were probably not caused by contamination from outside, since ammonia concentrations at all depths increased during the period March 26 to 31. The cause for the second ammonia increase is unknown.

Diatoms obviously remained unaffected by the growth of bacteria and organotrophic flagellates; this is indicated by their division rate which remained constant during the whole experiment (Fig. 1).

Uptake of inorganic nutrients

Except for ammonia, the measurable uptake of inorganic nutrients by *Skeletonema costatum* was not influenced by the growth of bacteria and organotrophic flagellates (Fig. 4 to 6). Fluctuations of phosphate and nitrate rates prior to definite uptake lie within the limits of measurement errors ($\pm$ 0.05 and $\pm$ 0.3 µmoles dm^{-3}, respectively). Silicate measurements showed a smaller relative error ($\pm$ 0.05 µmoles dm^{-3}), therefore the increase after March 23 (Fig. 5) is indicative of a first small uptake.

Phosphate and nitrate were taken up in demonstrable quantities only after the diatom growth had reached the high rate of about $2 \cdot 10^6$ cells d^{-1}. At 3 m a

final cell number of $35 \cdot 10^6$ diatoms dm^{-3} was obtained, not including burst or sedimented cells. Averaging uptake over the entire experiment gives values for mean nutrient consumption per 10^6 cells of: 0.02 µmoles o-phosphate; 0.32 µmoles nitrate; 0.21 µmoles silicate.

Apart from the early phosphate consumption, phosphate and silicate were taken up simultaneously, while the uptake of nitrate was clearly retarded. There is a strong indication that nitrogen sources other than nitrate were used first. According to mean nutrient consumption values per 10^6 cells, a diatom increase of $5.4 \cdot 10^6$ cells dm^{-3}, which occurred at 3 m on March 30 to 31, requires a nitrate uptake of 1.7 µmoles dm^{-3}. However, we recorded an uptake of only 0.3 µmoles dm^{-3} for this period.

Measurable nitrate uptake at 0 and 3 m was obtained only after ammonia concentrations had fallen below 0.4 µmoles dm^{-3}. This means that ammonia was used by *Skeletonema costatum* before nitrate was taken up – a finding reported for marine phytoplankton by several investigators (for review see McCarthy, 1980). Consequently, our calculated N:P uptake ratio of 16, resulting from the measured nitrate:phosphate uptake, is an underestimate, although it is in good accordance with Fleming (1940), Harris and Riley (1956) and Redfield (1958) who reported a ratio of 15–17:1 for phytoplankton. In our tank experiment cell composition should be roughly comparable with the uptake ratio due to the dominance of nutrient uptake by diatoms over other removal or release processes.

Dynamics of total dissolved carbohydrates

Initial carbohydrate concentrations

Relatively high initial concentrations of total dissolved carbohydrates (2.3 µmoles dm^{-3}, Fig. 8) were recorded in the filtered fjordwater enclosed after a diatom bloom, consisting largely of *Thalassiosira nordenskioeldii, Thalassionema nitzschioides* and *Chaetoceros debilis* (Brockmann et al., 1981; Jahnke et al., 1983; Kattner et al., 1983). The initial pool of dissolved carbohydrates may have consisted mainly of material originating in walls of dead cells. Because of the more complex composition of cell walls (Haug and Myklestad, 1976), their release products are believed to be less easily utilized than those from living cells. The possibility cannot be excluded that the initial high level of carbohydrate concentrations was caused in part by very small cell-wall fragments, passing 0.1 µm filters and therefore being measured by the tryptophansulfuric acid-method.

Diurnal release of carbohydrates from March 18 to 24

The daily cycle computed for the relatively small fluctuations of carbohydrate concentrations during the first 6 d of the experiment (Fig. 10), can be explained by photosynthetically active organisms, such as *Skeletonema costatum*, releasing carbohydrates during daylight which were probably taken up again by heterotrophic organisms. Cell numbers of *S. costatum* increased from $5 \cdot 10^4$ dm^{-3} to only $30 \cdot 10^4$ dm^{-3} from March 18 to 23 (Fig. 1) and were perhaps a little too low to account for the relatively high carbohydrate release during this period. Possibly, the release of organic substances facilitated a continuous increase of diatom cell numbers by chelating trace metals or by promotion of bacteria which release vitamin B_{12} required by *Skeletonema costatum* (Droop, 1955, 1962).

A mean daily release rate of 0.2 µmoles Glc Eq dm^{-3} was computed for the initial phase of the experiment at 3 m. This is equivalent to 2.0 pmoles per cell, decreasing to 0.4 pmoles $cell^{-1}$ with increasing cell numbers (29 to 144 pg C $cell^{-1}$). For *Skeletonema costatum* with a mean cell size of 6×9 µ a carbon content of about 25 pg $cell^{-1}$ was computed (Hagmeier, pers. comm.). If the diatoms were responsible for the increase in carbohydrate concentrations during daylight, they would have had to release 1.1 to 5.8 times their carbon content d^{-1} from March 18 to 23. Since healthy algae can release more than 40% of their photosynthetic products (Lewin, 1956; Prager et al., 1959; Guillard and Hellebust, 1971; Ignatiades and Fogg, 1973), such high release rates of *S. costatum* do not seem to be impossible.

Algae species other than *Skeletonema costatum* could not have been responsible for the high carbohydrate release during daylight, because of their absence from microscopic samples. Coulter Counter data gave no hint that small green flagellates occurred in larger numbers during the time in question. The supposition that cell fragments of fjord algae, present in the enclosure in spite of pre-filtration, released the carbohydrates, is contradicted by the fact that the daily carbohydrate release rates remained constant over the period of 6 d, whereas the activity of cell fragment enzymes is believed to diminish during such a long period.

The calculable release value for March 18 to 23 totals 1.1 µmoles Glc Eq dm^{-3} at 3 m and represents a high energy potential. The consumers in question are the organotrophic bacteria which showed their highest increase rates in counts during this time (Fig. 2).

Attention should be drawn to the fact that during the night carbohydrate concentrations never fell to values lower than 2.2 µmoles dm^{-3} (Fig. 8). The relatively

small fraction of carbohydrates released during daylight, amounting at most to 12 % of the total carbohydrates, showed a high turnover, whereas the large fraction of inert carbohydrates remained unchanged and constantly high.

Decrease of carbohydrate concentrations from March 23 to 24

The sudden, rapid decrease in carbohydrate concentrations from March 23 to 24 can be interpreted by assuming that bacteria, as a result of a succession of different species, or due to food limitation, adapted their enzyme systems to the degradation of complex and inert carbohydrates. Decreasing ammonia concentrations in the period March 23 to 25 (Fig. 7) indicate that carbohydrates were a main source of energy for organotrophic organisms, whereas free ammonia perhaps had to be used as a nitrogen source. Within 2 d, at least 1.1 µmoles Glc Eq dm^{-3} were taken up, with similar rates at all depths (Fig. 9).

Organotrophy among non-pigmented flagellates has been demonstrated at high solute concentrations (Droop, 1970; Haas and Webb, 1979), but is not a thoroughly studied subject. Haas and Webb (1979) tested 5 bacteria-grazing species of nanoflagellates and were unable to demonstrate uptake of 11 ^{14}C-labelled amino acids, carbohydrates and organic acids commonly present in sea water at concentrations far above their ambient levels. Considering the much lower surface to cell volume ratio, compared to bacteria, the generally wide spectrum of metabolic capabilities among bacteria, and their generally higher growth rates, the flagellates would seem to be non-competitive (Fenchel and Jørgensen, 1977). In our experiment, however, they may actively have removed carbohydrates by phagocytosis of particles, which, due to the filtration methods used, perhaps were included in the dissolved fraction. The rapid increase in 1.5 to 3 µm particle numbers (Fig. 3) began exactly when carbohydrate concentrations fell (Fig. 8).

Low carbohydrate concentrations from March 24 to April 1

Utilization of the fraction of complex and stable carbohydrates terminated on March 25 and did not include all carbohydrates. Values lower than 1.1 to 1.2 µmoles Glc Eq dm^{-3} were not measured during the whole experiment (Fig. 8) indicating a degradation limit, since the minimum concentration was reached within a short time and then remained constant.

The small fluctuations of carbohydrate concentrations shown in Fig. 8 for March 26 to April 1 cannot be interpreted as diurnal release. A combination of different processes must be assumed, e.g. release by growing diatoms and by different degradation processes, as well as uptake by heterotrophic organisms. In any case, a food limitation for organotrophic organisms is indicated for the whole period of March 26 to April 1 by the low carbohydrate concentrations.

High fluctuations of carbohydrate concentrations from April 1 to 5

The short-lived carbohydrate maxima in the period April 2 to 5, which were especially high during daylight or shortly after sunset, can only be attributed to release by photosynthetically active organisms such as *Skeletonema costatum*. The diatoms reached maximum cell numbers during this time, which was also when nutrients were exhausted. Similar fluctuations of release had been observed for amino acids and carbohydrates in 1973 tank experiments with cultures of *Thalassiosira rotula* (Hammer and Eberlein, 1981; Hammer et al., 1981; Eberlein, unpubl.).

Extremely high carbohydrate maxima at 10 m (Fig. 11), which appeared only at 12.00 h and 15.00 h, point to a release by photosynthetically active organisms, due to a surplus production during maximum light intensities. Since such high carbohydrate maxima were not found in upper water depths, high release rates cannot be attributed to a damage of cells by too high light intensities.

Since each high release of carbohydrates was immediately followed by a rapid decrease in concentration, highly efficient uptake processes must be taken into account. The organisms in question are organotrophic bacteria and flagellates; their numbers increased again a little at the end of the experiment (Fig. 2 and 3), although adsorption to particles or reuptake of the released organic substances by the diatoms themselves cannot be excluded (Hellebust, 1970; Wheeler et al., 1974; Sepers, 1977; Lewin and Hellebust, 1978). Adsorption to tank walls is unlikely to occur so rapidly.

Carbohydrate release at different water depths (Fig. 11) was influenced by varying diatom development with depth, due to different light conditions. At 3 m, the stationary phase was reached on April 3 (Fig. 1), when nutrients were used up (Fig. 4 to 6). At the surface, the stationary phase was reached 1 d earlier, as shown by nutrient uptake rates (Fig. 4 to 6), while nutrient concentrations at 10 and 15 m indicate that the stationary phase was not reached at all. Fig. 1 illustrates that the stationary phase at these depths did not begin before April 5.

The release of carbohydrates was different not only at different depths, but also during different growth phases. During exponential phase, background-level values of carbohydrate concentrations did not increase (Fig. 10, 10 m). This means that the concentrations of the complex and stable carbohydrate fraction remained constant, whereas they increased during the stationary phase (Fig. 11, 0 and 3 m), probably due to senescence and decomposition of some of the diatom cells. As stated above, carbohydrates released from cell walls are more complex and less easily utilized than storage saccharides released by living cells.

On April 1, 2, and 4 no high carbohydrate release rates could be demonstrated at any depth. It is not clear whether this is due to environmental or internal factors.

The total quantity of released carbohydrates was extremely high at the end of the experiment; however only up to 0.2 pmoles $cell^{-1}$ d^{-1} was released, which is less than 0.4 to 2.0 pmoles $cell^{-1}$ d^{-1} at the beginning of the experiment. The methods used in this investigation did not allow a determination of carbohydrates taken up immediately after release. Their concentrations could have increased towards the end of the experiment and could be missing in the estimations, resulting in an underestimate of the total release per cell. It is possible that organotrophic bacteria were living directly on the surface of diatom cell walls taking up most of the liberated substances during exudation.

CONCLUSIONS

Dissolved carbohydrates in the enclosure experiment originated from different sources. One part was in the water by the beginning of the experiment, probably due to a phytoplankton bloom in the fjord before the test water was filtered and enclosed. Another part was released by growing *Skeletonema costatum*. The release per cell was initially extremely high, but maximum concentrations were released at the end of the exponential phase. During the course of a day, release activity changed strongly within a few hours. Main release occurred from noon to midnight.

At the start of the experiment, before succession or adaptation of bacteria began, the maximum portion of rapidly utilizable carbohydrates reached only 12 % of total carbohydrates. At the end of the exponential phase of *Skeletonema costatum*, however, the maximum value of this fraction amounted to 82 % (at 10 m). During the stationary phase the labile portion of carbohydrates, which showed a high turnover, decreased again to a lower maximum value of 37 % (at 0 m). Obviously, organotrophic bacteria and perhaps other microorganisms may have utilized the dissolved carbohydrates in a different way, according to their origin. Presumably, those carbohydrates were taken up immediately that were released by healthy cells during the exponential phase and at the beginning of the stationary phase. Carbohydrates only utilized after adaptation or succession of bacteria and those not taken up at all, originated probably from senescent and decomposing phytoplankton cells.

According to the different utilization types of carbohydrates, their concentrations decreased to 2 different ground levels. During the first 6 d of the experiment, when only those carbohydrates were consumed which were released in a daily cycle, a background level of 2.2 μmoles Glc Eq dm^{-3} was reached by uptake processes. As a result of bacteria succession or adaptation a second background level of 1.2 μmoles Glc Eq dm^{-3} was attained on March 27. Only at the end of the experiment did these background-level concentrations increase again slightly at 0 and 3 m, probably due to senescence and decomposition processes at the beginning of the stationary phase of the diatoms.

We plan further work in order to obtain more information on the highly efficient uptake processes during enclosure. Efforts will also be directed at determining release and uptake rates of carbohydrates more exactly by employing other methods, and at finding out which events in diatom cell growth and metabolism caused the extremely high but short-term carbohydrate release maxima in the end of the exponential and the beginning of the stationary phase.

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