Are bacteria active in the cold pelagic ecosystem of the Barents Sea ?

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Bacterial biomass and activity indicators have been studied at low water temperatures (−1.9 to +4°C) in the Barents Sea. Strong responses by indicators of bacterial activity, such as hydrolytic enzyme and substrate uptake potentials, were observed in association with the development of phytoplankton blooms. At late successional stages of blooms, observation by epifluorescence microscopy revealed heavy bacterial colonisation of detrital matter, in particular of senescent colonies of *Phaeocystis pouchetii*. Based on the retention of bacteria on filters of 1 µm pore size, up to 55% of the bacterial population was estimated to be attached to organic aggregates in some cases. Based on thymidine incorporation and a conventional conversion factor, bacterial generation times as short as one day were estimated at temperatures below zero. Changes in substrate availability governed by the successional stages of the planktonic ecosystem seem to be more important as controlling factors for bacterial growth than the low temperatures of the Barents Sea.

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Introduction

Pelagic food webs are dynamic systems where both transient responses and equilibrium states depend upon the interactions between a large number of processes. If these processes differ in temperature sensitivity, the balance between them may shift, and, as a result, food webs in polar regions may theoretically behave differently than those of temperate regions. One such mechanism was proposed by Pomeroy & Deibel (1986) when considering bacterial degradation of organic material. Based on data indicating low bacterial activity below +4°C, they made the suggestion that bacterial processes are of less importance in cold than in temperate waters. One of the significant aspects of such a mechanism is the potential effect of temperature on the relative importance of bacterial dissolution/degradation of particulate matter, versus metazoan feeding on these particles. With the large variation expected in time and space for both of these processes, and with the technical difficulties associated with their measurement, a direct comparison of the relative importance of these two processes for different regions is not easily obtained. If, however, bacterial growth in cold waters is severely inhibited, phytoplankton blooms in cold waters would not be expected to be followed by any strong responses in bacterial biomass or activity. A predominance of metazoan consumption prior to bacterial invasion of detrital particles would also make the observation of colonised particulate material unlikely.

In the polar and meltwater regions of the Barents Sea, phytoplankton blooms occur at water temperatures close to freezing point (Skjoldal & Rey 1989), and the subsequent processes of DOM and detrital POM transformation must also occur at this low temperature. These blooms are often dominated by the colony forming prymnesiophyte *Phaeocystis pouchetii* (Skjoldal & Rey 1989). In temperate areas, *Phaeocystis* blooms have been shown to leave large amounts of organic material in the water (Eberlein et al. 1985), and after a delay of 5–10 days, the blooms are followed by a development of planktonic bacteria (Billen & Fontigny 1987). If low-temperature inhibition of bacterial degradation is important, one would expect bacterial responses to *Phaeocystis* blooms in the Barents Sea to be feeble, more delayed or even absent. Processes related to the bacterial dissolution and degradation of particles involves the hydrolysis of macromolecules, the uptake of monomers, and the formation of bacterial biomass. We have found that bacterial abundance, activity poten-

tials for hydrolytic enzymes and uptake potentials for monomers, as well as the incorporation rate of thymidine, all increase in response to phytoplankton blooms occurring at temperatures between −1 and +4°C in the Barents Sea. In senescent blooms, massive bacterial invasion of colonies was also observed. Little support was therefore found for a bacterial response qualitatively different from that described in temperate waters.

Materials and methods

Sampling

Samples were collected by a rosette sampler with Niskin water bottles attached to the CTD sonde, or, when large volumes were required, by 30L Niskin bottles shortly after the CTD cast. Geographical position of stations are given in Fig. 1. The stations are from different cruises: Stations 14, 24, 31, 52 are from cruises with coast guard vessels K/V ANDENES and K/V SENJA in April 1986. Stations 733, 890 and 961, with R/V G. O. SARS in June 1984, August 1985 and June 1987, respectively. Temperature dependence of proteases was measured on a sample of brown ice collected in April 1986 and thawed at an ambient temperature of 10°C. Incubations where performed at close to in situ temperature in a thermostatted water cooled incubator, or, for temperature dependence studies, in a temperature gradient incubator described by Eriksen & Goksøyr (1976).

Microscopic counts

Heterotrophic and autotrophic pico- and nanoplankton were counted by epifluorescence microscopy according to Martinussen & Thingstad (1991). Due to a double staining with primuline and DAPI and the alternation between blue and UV-exitation in the epi-fluorescence microscope, this procedure allows identification of autotrophs by chlorophyll autofluorescence, positive identification of cells based on the existence of a DAPI-stained nucleus, and the visualisation of the whole

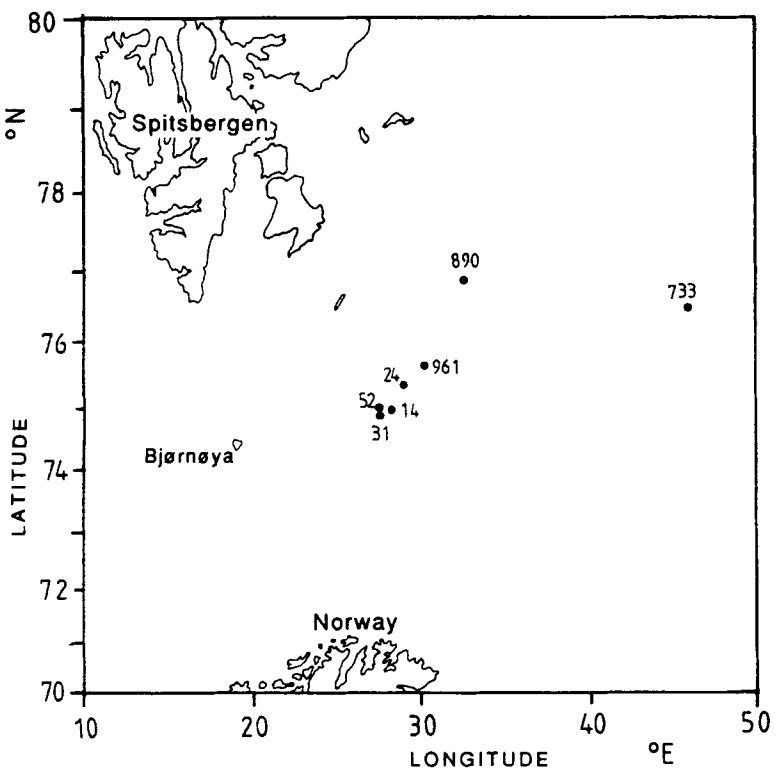


Fig. 1. Positions of stations in the Barents sea.

cell by the primuline stain. The primuline stain also allowed the visualisation of the mucus of *Phaeocystis* colonies. Samples were fixed with 2.5% final concentration of borate-buffered formalin. As a standard procedure, a 5 ml sample was filtered on a 0.2 µm pore-sized polycarbonate filter for bacterial counts and between 15 and 50 ml, dependent upon expected population density, on a 1 µm filter for counting of eucaryotes and retained bacteria. Bacterial cell volumes were calculated from linear dimensions determined in the microscope at 1000× magnification using a calibrated graticule (New Porton G12, Graticules Ltd., England) in one ocular.

Enzymatic activity potentials

Activity potentials for protease and β-glucosidase were determined according to Somville & Billen (1983) and Somville (1984) using a Perkin-Elmer LS5 fluorescence spectrophotometer.

Thymidine incorporation

Thymidine incorporation into cold TCA-precipitable material was determined according to Fuhrman & Azam (1982) modified by increasing the concentration of added thymidine to 10 nmole l^{-1}. Conversions from thymidine incorporated to estimates of cell production rates are based on a conversion factor of 2.4 10^{18} cells mol^{-1} as suggested by Fuhrman & Azam (1982) for offshore waters. Generation times are computed as ln2 × cell density (cell production rate)$^{-1}$.

Uptake potentials of ^{14}C-labelled glucose and amino acids

Uptake potentials for monomers were measured by adding 37 kBq of ^{14}C-labelled glucose (Amersham CFB.96) or a ^{14}C-labelled amino acid mixture (Amersham CFB.104) to 10 ml samples of seawater. Final concentrations of glucose and amino acids were 0.37 and 0.38 µmol l^{-1}, respectively. The different amino acids in the mixture had different specific activities, and conversion from DPM to nmoles are based on mean specific activity of the mixture (68 $\overline{\text{Bq}}$ · $mmol^{-1}$). After 5 h incubation, the samples were filtered on 0.2 µm cellulose-nitrate membrane filters. The filters were washed twice with filtered seawater, dried, and counted by scintillation counting.

Chlorophyll, NO_3, in-situ fluorescence and light

These data were obtained from the sampling programme of the Marine Research Institute, Bergen. Chlorophyll *a* concentrations were measured fluorometrically after acetone extraction of filtered samples, nitrate was measured by autoanalyser as described in Føyn et al. (1981), fluorescence profiles were obtained using a submersible fluorometer (Q-fluorometer). An exception to this is Station 961, where profiles of in situ fluorescence, light, and σ_t profiles are obtained from B. G. Mitchell, Scripps Institution of Oceanography, and were collected using a bio-optical rig (Biospherical Instruments) equipped with sensors MER-12, MER-08, and MER-53.

Results

Ice-edge bloom, April 1986

The bacterial development during the first phase of the ice-edge bloom was followed on a cruise

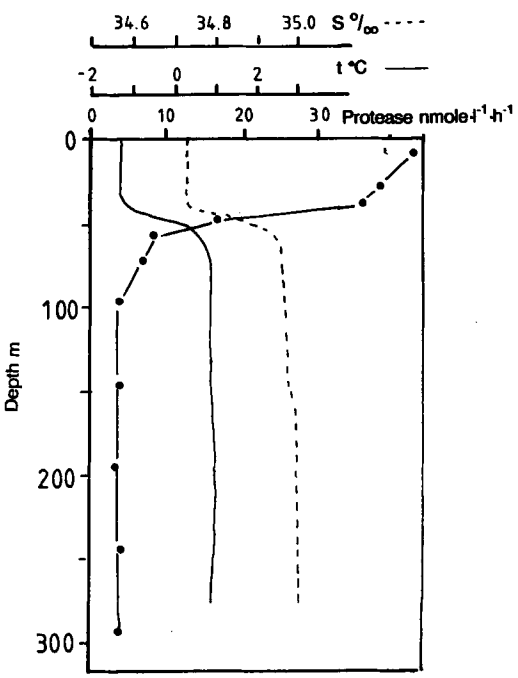


Fig. 2. Station 31, April 1986. Profiles of salinity (broken line), temperature (solid line), and protease activity potential (solid circles). Development of bacterial activities occurring in the cold meltwater layer on top of warmer Atlantic Water masses.

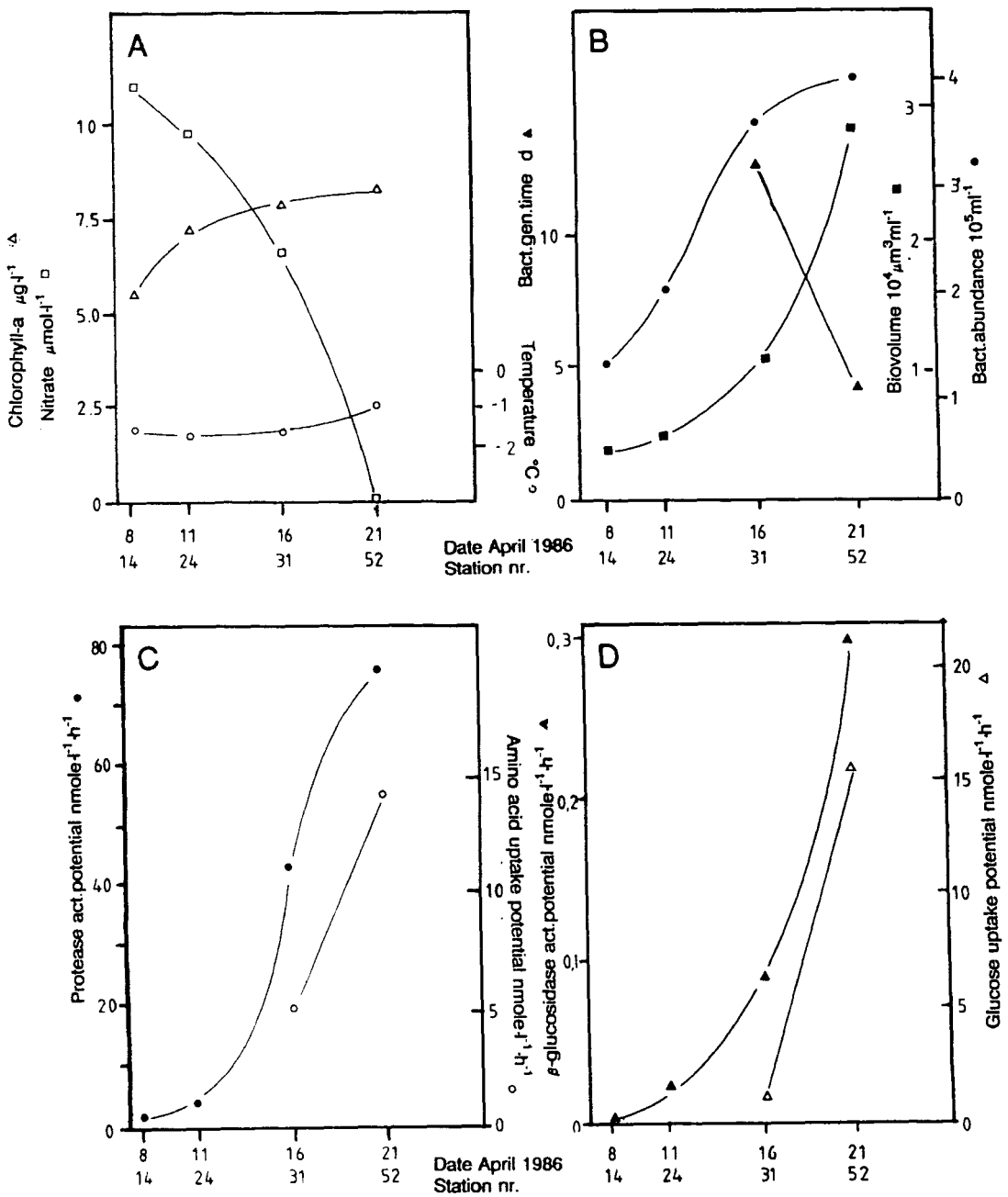


Fig. 3. Sequence of stations during ice-edge bloom. Samples from 10 m depth. A. Chlorophyll *a* (open triangles), NO_3 (open squares), and temperature (open circles). B. Bacterial abundance (solid circles), bacterial biovolume (solid squares), and generation times estimated from thymidine incorporation (solid triangles). C. Protease activity potential (solid circles), uptake potential for amino acids (open circles). D. β-glucosidase activity potential (solid triangles), and glucose uptake potential (open triangles).

with coast guard vessel K/V ANDENES in April 1986 in the area west of Sentralbanken in the Barents Sea (Fig. 1). The meltwater layer in the area extended down to about 50 m (Fig. 2). Return of the ship to the same area with intervals of a few days allowed the ordering of samples in a time sequence spanning 13 days, permitting an indication of how the bloom in the area developed, although no strict time sequence of the development in a well-defined water body was possible. During the period of observation, the increase in chlorophyll levelled off, and nitrate was consumed. A slight heating of the meltwater layer occurred, with water temperatures at 10 m reaching −1.0°C at the end of the period (Fig. 3A). Bacterial abundance increased by a factor of 3 in numbers and 5.6 in total biovolume (Fig. 3B), the main increase in bacterial biovolume occurred at the end of the period.

All measurements of bacterial activity indicated a transition to a phase of active bacterial growth at the end of the period. The enzymatic activity potentials increased throughout the period with factors of 60 and 40 for β-glucosidase and protease respectively. Due to a temporary failure of the scintillation counter, uptake measurements are only available for Stations 31 and 52, but glucose and amino acid uptake potentials both showed the same rapid increase as the enzyme activity potentials (Fig. 3C, D). The bacterial response was restricted to the cold water layer with no response in the warmer Atlantic Water below as illustrated by the profile of protease activity potential in Fig. 2. Estimates of bacterial generation times from thymidine incorporation indicated a decrease in mean generation time of the bacterial population from 13 to 4 days between the 8th and the 13th day of the period (Fig. 3B).

Subsurface chlorophyll maximum

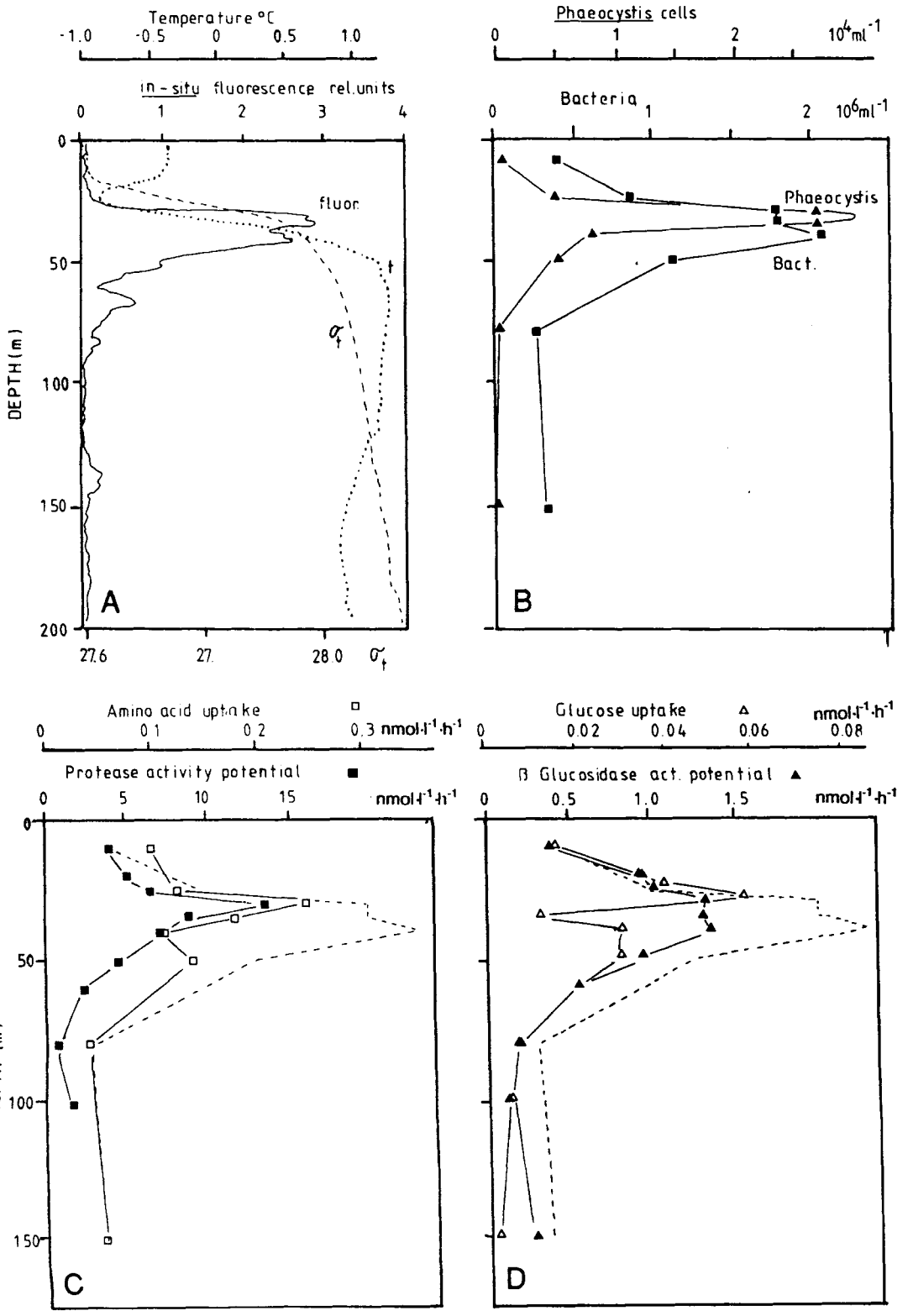
Station 961, in the central Barents Sea, June 1987, is an example of a later successional stage where a sharp subsurface chlorophyll *a* maximum has developed at the pycnocline (Fig. 4A) between the meltwater layer and the underlying Atlantic Water. The autotrophic community was completely dominated by *Phaeocystis*. The profile of bacterial abundance had a sharp peak very similar to the distribution of *Phaeocystis* cells (Fig. 4B), but with a maximum of $2 \cdot 10^6$ ml^{-1} shifted slightly downwards in the water column relative to the peak in abundance of *Phaeocystis*. Activity potentials of hydrolytic enzymes and uptake of glucose and amino acids all indicated the same type of depth profiles with peaks associated with the upper part of peak in bacterial abundance (Fig. 4C, D). At this station, colonies of *Phaeocystis* were still relatively free of bacteria, and maximum retention of bacteria on 1 μm filters was 10% at 10 m. At the fluorescence maximum 3% were retained.

Bloom in water without well-defined pyknocline

Station 733 in the eastern Barents Sea, June 1984, represents a situation with temperatures below zero at all depths. There was no distinct pycnocline, but a gradual increase in σ_t downwards. (Fig. 5A). The in situ fluorescence increased down to 20 m which corresponded to the bottom of the photic zone. A broad maximum in the fluorescence profile extended down to about 60 m with a long "tail" extending to the lowest measured depth at 90 m. Chlorophyll at the depths of maximum fluorescence corresponded to 3.8 μg Chl · l^{-1}. Estimates of bacterial generation time had two minima, 1.2 and 1.5 days, at the surface and at 30 m respectively (Fig. 5B). Heavily invaded *Phaeocystis* colonies were observed and bacterial retention on 1 μm reached a maximum of 40% at 30 m depth (Fig. 5B).

Post-bloom situation. August 1985

Station 890 in the central Barents Sea, August 1985, represents a situation with low values of in situ fluorescence throughout the water column, combined with nutrient depletion above the pycnocline (Fig. 6A). Except for a maximum of 0.9 μg Chl · l^{-1} at 20 m, values were below 0.4 μg Chl · l^{-1}. Water temperature above the pycnocline at 10 m were +4°C, decreasing to −1.8 in the underlying polar waters. The phytoplankton community was dominated by a small, unidentified picoplanktonic species approximately 1.5 μm in diameter and by *Dinobryon* spp. The fastest bacterial growth was found at the bottom of the pycnocline (20 m) (Fig. 6B) where a bacterial generation time of 1.0 d was estimated. At all depths of this station, aggregates with bacterial colonisation were observed, a feature particularly prominent at 20 m. This was reflected in a high fraction (55%) of the bacterial population being retained on 1 μm polycarbonate filters. The extreme maximum of bacterial retention in the


Temperature °C
-1.0 -0.5 0 0.5 1.0
in-situ fluorescence rel.units
0 1 2 3 4
fluor
σ_t
DEPTH (m)
0 50 100 150 200
27.6 27. 28.0 σ_t
A
Phaeocystis cells
0 1 2 $10^4 ml^{-1}$
Bacteria
0 1 2 $10^6 ml^{-1}$
Phaeocystis
Bact.
B
Amino acid uptake
0 0.1 0.2 0.3 nmol·l⁻¹·h⁻¹
Protease activity potential
0 5 10 15 nmol·l⁻¹·h⁻¹
C
Glucose uptake
0 0.02 0.04 0.06 0.08 nmol·l⁻¹·h⁻¹
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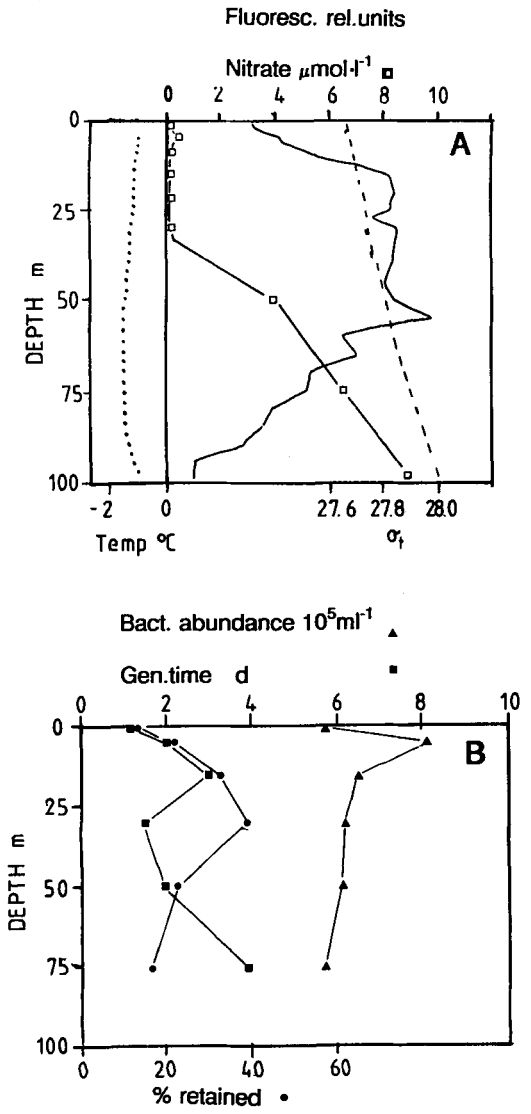


Fig. 5. Bloom in water without well-defined pycnocline. Station 733, June 1984. A. Profiles of fluorescence (solid line), temperature (dotted line), σ_t (broken line) and nitrate (open squares). B. Bacterial abundance (solid triangles), generation time estimates (solid squares), and percentage of bacterial population retained on 1 µm polycarbonate filters (solid circles).

vicinity of the pycnocline was specific for this station. The origin of the organic aggregates at this station is unknown. At other stations, however, senescent colonies of *Phaeocystis pouchetii* heavily colonised by bacteria were easily recognisable. Classification of the water samples according to whether the phytoplankton community was dominated by *Phaeocystis*, the unidentified picoplankton sp., a mixture of these, or dominated by other species demonstrated a clear correlation between the *Phaeocystis*/picoplankton communities, and a high retention of bacteria by 1 µm filters (Fig.7).

Temperature sensitivity of protease

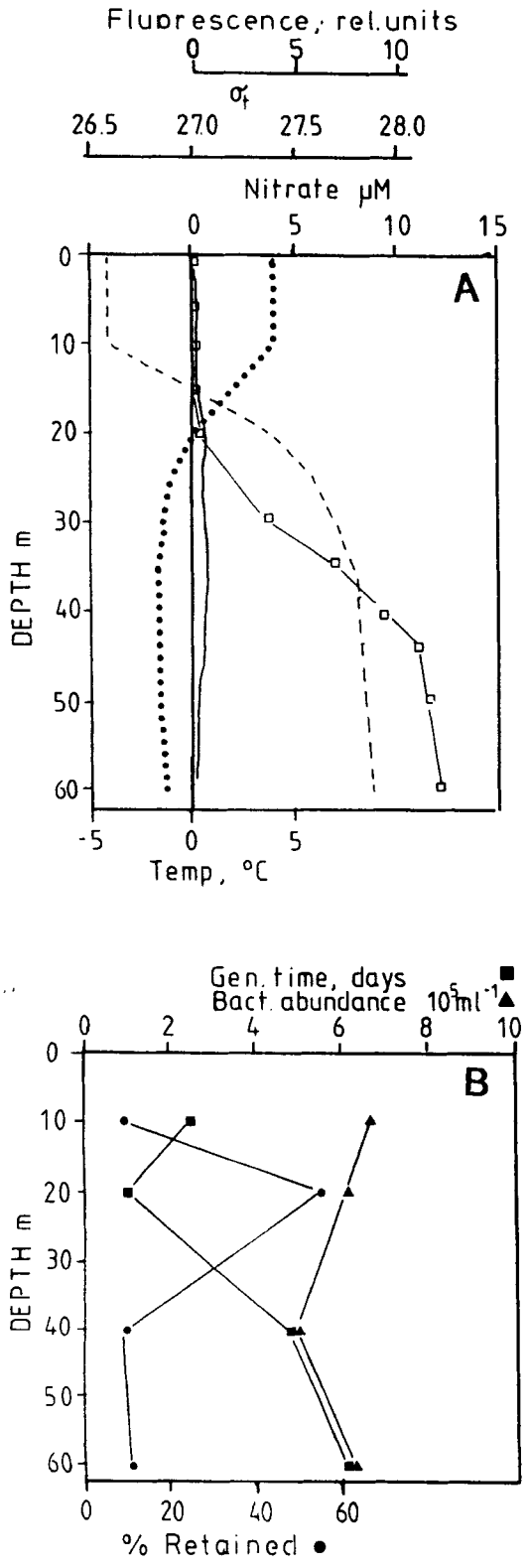
The Arrhenius plot for hydrolysis of the artificial substrate by the natural mixture of proteases in brown sea ice was linear ($R^2 = 0.996$) in the interval − 1.5 (lowest temperature investigated) to 21°C (Fig. 8). The slope of the linear part corresponds to an activation energy of 12.1 kcal · mole^{-1} which corresponds to a Q_{10} in the range −1.5 to 8.5°C of about 2.2.

Discussion

Bacterial abundances and activities, not drastically different from those of temperate waters, may develop in the Barents Sea, even at subzero temperatures. This is a conclusion in accordance with results previously reported by investigators in the Antarctic Ocean (Hodson et al. 1981; Hanson & Lowery 1985). We found, however, a large range in values of thymidine uptake, from less than 0.004 nmole · l^{-1} · day^{-1} in samples taken in late winter before the onset of the ice edge bloom to 0.26 nmole · l^{-1} · day^{-1} in connection with deep chlorophyll maxima in August.

Our sampling did not allow a strict determination of the timing between the peak of the ice-edge phytoplankton bloom and the bacterial response. The sequence of stations in April 1986 (Fig. 3) did, however, suggest that the response was initiated even before the exhaustion of the nitrate. No indications were found of a delay substantially longer than the 5–10 days reported for temperate waters by Billen & Fontigny (1987). At later stages of the bloom, a close correlation between the profile of bacterial abundance and the deep fluorescence maximum could be observed (Fig. 4), with bacterial abundances up

Fig. 4. Deep fluorescence maximum, station 961, June 1987. A. Fluorescence (solid line), temperature (dotted line) and σ_t (broken line). B. Cell counts of *Phaeocystis* (solid triangles), and bacteria (solid squares). C. Protease activity (solid squares) and amino acid uptake (open squares) potentials. D. β-glucosidase activity (solid triangles), and glucose uptake (open triangles) potentials.



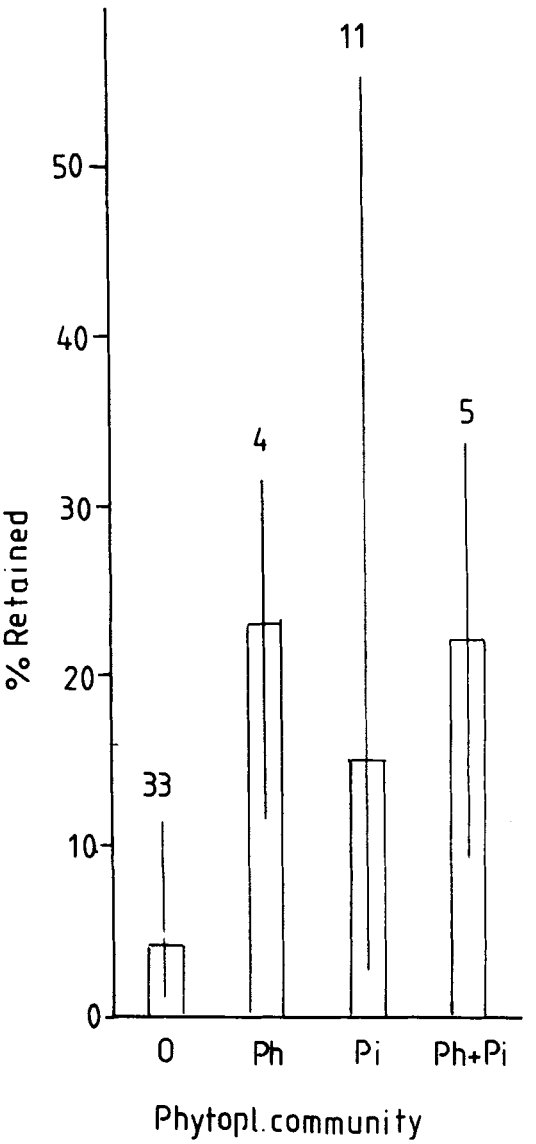


Fig. 7. Retention of bacteria on 1 µm polycarbonate filters. Water samples from cruise in August 1985 classified according to dominance of phytoplankton community by *Phaeocystis* (Ph), picoplankton (Pi), a combination of these (Ph + Pi), or by other groups (O). Bars and vertical lines indicate mean and range, respectively. Number of water samples in each category given above each bar.

Fig. 6. Post-bloom situation, Station 890, August 1985. A. Profiles of fluorescence (solid line), temperature (dotted line), σ_t (broken line), and nitrate (open squares). B. Bacterial abundance (solid triangles), generation time estimates (solid squares), and retention on 1 µm polycarbonate filters (solid circles).

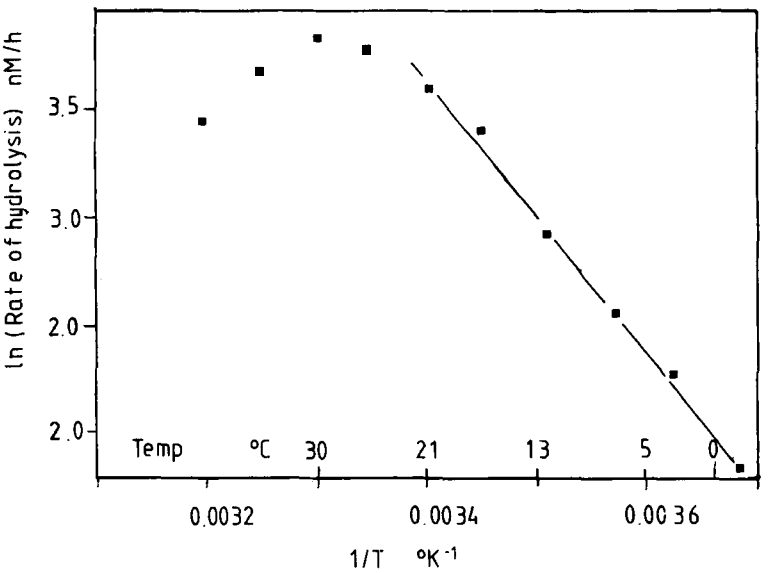


Fig. 8. Arrhenius plot of the rate of hydrolysis of L-leucyl-β-naphtylamide by proteases from a sample of brown ice.

to $2 \cdot 10^6$ ml^{-1}, similar to levels observed in coastal temperate waters (Azam et al. 1983). Our generation time estimates are based on a conversion factor between thymidine incorporation and cell production rate which is not determined for the actual environment(s). Since the factor is known to vary (Fuhrman & Azam 1982; Riemann et al. 1987), our generation time estimates must be taken as indicative only. Some of the higher values obtained for the conversion factor seem, however, to be associated with the addition of thymidine at concentrations below 10 nmole $\cdot$ l^{-1}, while lower values have been determined in coastal waters (Riemann & Bell 1990). The fastest growth with generation times about 1 day are, however, comparable to generation times estimated in temperate waters. As an example, Riemann et al. (1984) report values based on thymidine uptake between 17 and 91 hours through a diel cycle in a Danish coastal environment. In laboratory studies, Harder & Veldkamp (1971) reported a maximum growth rate of 0.073 h^{-1} at −2°C for one of the species investigated (*Pseudomonas* L12), corresponding to a generation time of 9.5 hours. This is below the generation times estimated by us, and demonstrates the feasibility at low temperatures of generation times that are short when viewed in an ecological context. The maximum protease activity potential observed at Station 52 (Fig. 3C) was 1.1 nmole $\cdot$ l^{-1} $\cdot$ min^{-1}. Using the temperature dependence determined, this corresponds to an activity of about 3 nmole $\cdot$ l^{-1} $\cdot$ min^{-1} around 15°C. This is comparable to typical values reported by Fontigny et al. (1987) for coastal seawater in the North Sea, although maximum values found in this more eutrophic environment exceeded 20 nmole $\cdot$ l^{-1} $\cdot$ min^{-1}.

From the visual observation of how bacterial microcolonies had formed on senescent *Phaeocystis* colonies, we felt fairly convinced that the colonisation was accompanied by active growth of attached bacteria. We have, however, no estimate of the relative proportion between those bacteria that were primary invaders and their offspring on the mucous material. In situations with high mucus content of the water, the retention of bacteria by 1 µm filters may overestimate the proportion of attached bacteria due to entrapment of bacteria in the mucus during the filtration process. The observation of colonies without attached bacteria during early stages of the blooms do, however, show that fresh mucus did not collect bacteria during the filtration process. While we feel confident that our observation shows that detrital particulate matter is colonised, we can not determine to what degree the material had been respired. A possible difference in the temperature responses of growth and respiration has been suggested (Christian & Wiebe 1974). A large temperature dependence for respiration could imply a large change in consumption rate

of organic matter, without this being revealed in measurements related to changes in bacterial biomass.

Temperature has been shown to influence most of the important growth parameters for bacteria. In a study of *Aerobacter aerogenes* (25 to 40°C), Topiwala (1971) found that parameters such as the half saturation constant for growth and endogenous metabolism were temperature dependent. Harder & Veldkamp (1967) found that a decrease in temperature was compensated for by an increase in the concentration of RNA and respiratory enzymes. Bacterial degradation of organic matter within an ecosystem context is, however, not simply a function of the physiological properties of the bacteria; degradation may also be strongly controlled by trophic interactions such as competition, predation, and remineralisation (Pengerud et al. 1987), and therefore by the temperature dependence of such processes. In a system where bacterial growth rate is controlled by organic or inorganic substrate limitation, moderate changes in maximum growth rate with temperature may be unimportant or compensated for by the dynamics of the system. An example is perhaps a possible compensation for low temperature by high substrate concentrations as suggested by Pomeroy & Wiebe (1988). Supporting evidence for this was found in investigations of amino acid concentrations and bacterial activity in the arctic (Pomeroy et al. 1990).

If extracellular hydrolysis is the rate limiting step of macromolecular degradation as suggested by Billen (1988), the temperature dependence of this process is particularly important. The extension of the linear part of the Arrhenius plot for hydrolysis by Barents Sea proteases to temperatures below 0°C (Fig. 8) demonstrates that enzymes functioning normally at low temperatures have developed in this ecosystem. The existence of bacteria with a complete enzymatic machinery functioning normally at low temperatures has been demonstrated two decades ago by Harder & Veldkamp (1971) who studied a group of *Pseudomonas* spp. for which the linear part of the Arrhenius plot for maximum growth rate extended down to between −4 and −5°C. Q_{10} values reported for enzyme catalysed reactions in natural systems seem to be fairly uniform. The value of about 2.2, corresponding to an activation energy of 12.1 kcal · mole^{-1}, found for hydrolysis by Barents Sea proteases, can be compared to the value 12.0 kcal · mole^{-1} reported for hydrolysis of casein with trypsin (White et al. 1968), to the value of 11 kcal · mole^{-1} reported for maximum growth rate of both obligate and facultative psychrophilic bacteria (Harder & Veldkamp (1971), or to a range of Q_{10} values between 1.8 and 2.3 for photosynthetic capacity of natural phytoplankton as reviewed by Harris (1980).

Any extreme sensitivity of bacterial processes to low temperatures of the type suggested by Pomeroy & Deibel (1986) or Pomeroy & Wiebe (1988) does therefore not seem to be of universal validity or to be a necessary consequence of any fundamental limitations in bacterial physiology. The reason for this apparent discrepancy in results is not immediately obvious. One may speculate on the possibility that there are differences in the composition of the bacterial community in permanently and in seasonally cold waters. Polar Water masses in the Barents Sea are characterised by temperatures below 0°C. The Atlantic inflow has a mean of 6.2°C, but this decreases rapidly east and northwards into the Barents Sea (Loeng 1989), and the Barents Sea therefore classifies as a permanently cold environment. In a one-year study in the Baltic when surface temperatures varied from 0 to 14°C, Kuosa & Kivi (1989) found a peak in bacterial production following the spring phytoplankton bloom in a period during which water temperatures increased from 2 to 6°C. This was a result seemingly in qualitative accordance with our results from the Barents Sea. In experiments with water collected in April from the same area, however, bacterial growth was not found when filtered samples were incubated at in situ temperature (+1°C), although higher incubation temperatures induced rapid bacterial growth (Autio 1990), suggesting that cold-adapted bacteria were not present. A unified picture of how the bacterial community adapt to temperature in permanently and in seasonally cold environments seems therefore still to be lacking.

Ecosystem behavior is a result not only of the interactions between biological processes but also of the interactions between biological and physical processes. Perhaps the latter is a field where differences in temperature dependence of interacting rates is more likely to be expected. As an example, viscosity of 35‰ seawater decreases by a factor of only 1.36 between 0 and 10°C (Knauss 1978). One may speculate whether this response is sufficiently small to shift the balance between sedimentation and degradation rate of particles

and, as a result, influence the degree of nutrient impoverishment of the meltwater layer or the amount of organic material reaching the sediments. Such a shift would constitute a third element to be considered in the discussion of whether a match in time between the spring phytoplankton bloom and the development of mezozooplankton is important for the partitioning of the primary production between sedimentation and predation by metazoans in the Barents Sea ecosystem (Rey et al. 1987; Wassmann 1989). It would also effect the carbon budget of the upper ocean.

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