ELSEVIER

Journal of Marine Systems 38 (2002) 77–91

JOURNAL OF MARINE SYSTEMS

www.elsevier.com/locate/jmarsys

Distribution of bacterial biomass and activity in the marginal ice zone of the central Barents Sea during summer

M.H. Howard-Jones [a,b,*], V.D. Ballard [c], A.E. Allen [a,d], M.E. Frischer [a], P.G. Verity [a]

[a] *Skidaway Institute of Oceanography, University System of Georgia, Ocean Science Circle 10, Savannah, GA 31411, USA*
[b] *Department of Biology, Georgia Institute of Technology, Atlanta, GA 30332, USA*
[c] *Marine Sciences, Savannah State University, Savannah, GA 31404, USA*
[d] *Department of Ecology, University of Georgia, Athens, GA 30602, USA*

Received 22 August 2001; accepted 9 May 2002

Abstract

The purpose of this study was to determine bacterioplankton abundance and activity in the Barents Sea using the novel modified vital stain and probe (mVSP) method. The mVSP is a protocol that combines DAPI and propidium iodide staining with 16S rRNA eubacterial-specific oligonucleotide probes to determine the physiological status of individual microbial cells. Bacterial abundance and metabolic activity were measured in near-surface waters and with depth at stations in the central Barents Sea during a cruise in June/July 1999. Viral abundance was also determined for 19 transect stations and at depth (2–200 m) for five intensive 24-h stations. In general, bacterial and viral abundances varied across the transect, but showed peaks of abundance (6×10^9 cells l^{-1}, 9×10^9 viruses l^{-1}) in Polar Front water masses. Viruses were abundant in seawater and exceeded bacterial abundance. Metabolic activity was determined for individual cells using 16S rRNA eubacterial-specific oligonucleotide probes, and for the total community with ^{3}H-leucine incorporation. Activity measured by oligonucleotide probes increased from south to north. The fraction of cells that were active was lowest in the southern Barents Sea (20%) and highest in the Polar Front (53%). The proportion of cells at the 24-h stations that were determined to be active decreased with depth, but not with distance from ice cover. Leucine incorporation rates varied significantly and did not always correlate with probe measurements. The proportion of total cells that had compromised membranes and were therefore considered dead remained relatively constant (<10%) across the transect. The percent of dead cells in the near surface waters and at depth were statistically similar. The percent dead cells made up only a small fraction of the total population at the 24-h stations. The largest and most variable fraction of cells were those classified as low activity (25–80%), which supports the hypothesis that a significant fraction of cells in aquatic ecosystems are inactive. Bacterioplankton production rates ranged from <0.05 to 2.8 mg C m^{-3} day^{-1}. Growth rates ranged from <0.05 to 0.25 day^{-1}, implying turnover rates of 2.5 to >200 days. Our results demonstrate that bacterioplankton and viruses are dynamic but ubiquitous features of Arctic microbial communities. The contribution of bacteria and viruses to Arctic food webs is discussed.

Keywords: Bacteria; Activity; Viruses; Leucine; Probes; Barents Sea; Arctic

* Corresponding author. Department of Biological Sciences, Idaho State University, Pocatello, ID 83209, USA. Tel.: +1-208-282-4831; fax: +1-208-282-4570.

E-mail addresses: howamich@isu.edu (M.H. Howard-Jones), peter@skio.peachnet.edu (P.G. Verity).

0924-7963/02/$ - see front matter © 2002 Elsevier Science B.V. All rights reserved.
PII: S0924-7963(02)00170-7

1. Introduction

For many decades, it was thought that classical food chains dominated the worlds' oceans and bacteria were relatively unimportant components of marine planktonic food webs. However, studies have shown that bacteria are more abundant than previously thought, and can be responsible for a significant fraction of the primary production in the world's ocean (Azam et al., 1983; Stockner and Anita, 1986; Ducklow and Carlson, 1992; Fuhrman and Suttle, 1993). In the last 10 years, increased attention has been paid to the primary productivity of the polar regions and the potential role these regions may play in sequestering CO_2 (Walsh, 1989; Smith et al., 1995). Investigations reveal that some of the highest primary production rates in the worlds' oceans can be found in Arctic regions such as the northern Bering Sea, southern Chukchi Sea (Sambrotto et al., 1984) and Barents Sea (Slagstad and Wassmann, 1997; Olsson et al., 1999; Luchetta et al., 2000).

The biology of large Arctic phytoplankton and copepods has been studied extensively in the last century (Gran, 1902; Ussing, 1938; Smidt, 1979), but these studies measured bulk community properties of a very limited number of trophic links and, therefore, underestimated the complexity of the food web, specifically the part of the bacterial community (Pomeroy, 1974; Azam et al., 1983). Although the role that bacteria play in these cold environments continues to be unresolved, Arctic bacterioplankton appear to be important consumers of dissolved organic matter (Cota et al., 1990; Thingstad and Martinussen, 1991; Rivkin et al., 1991). It is well recognized that bacteria play a key role in the transformation of organic matter in aquatic ecosystems, yet little is known about the activity and viability of the cells participating in these transformations.

Investigations of bacterial communities have often been limited by methodologies. However, advances in direct enumeration of cells by fluorescent microscopy (Hobbie et al., 1977; Kepner and Pratt, 1994), and radiotracer methods for estimating community activity (Kirchman et al., 1985, 1986) have permitted more accurate examination of bacteria cells in aquatic ecosystems. One of the most widely used approaches for examining single-cell activity is fluorescence in situ hybridization (FISH), which determines cellular ribosomal content using fluorescently labeled oligonucleotide probes. Data from FISH investigations indicate that marine microbial communities can be characterized by at least three categories of cells: (1) dead cells that play no active or potential role in the cycling of elements, representing particulate organic carbon; (2) viable and active cells that play a functional role and participate in the production of biomass at the time of sampling; and (3) viable but inactive cells that might play a role in the future.

The purpose of this study was to employ the modified vital stain and probe method (mVSP) to determine the abundance and activity of bacterioplankton along a transect that started in the Atlantic water of the southern Barents Sea, crossed the Polar Front and ended in the marginal ice zone (MIZ) of the Arctic Ocean. The mVSP protocol investigates the physiological status of cells by employing the vital stain propidium iodide (PI), 16S rRNA oligonucleotide probes and the DNA stain DAPI (Howard-Jones et al., 2001). The modified method overcomes a limitation of the original method (Williams et al., 1998) by combining PI staining and probe hybridization into one, rather than two separate protocols. The mVSP simultaneously interrogates individual bacterial cells with all three components (DAPI, PI, probes). In addition to VSP measurements, viral abundance and leucine incorporation were examined with the ultimate goal of providing a better understanding of the contribution of bacterioplankton to Arctic food webs.

2. Materials and methods

2.1. Bacterioplankton samples

Near-surface water samples (2 m) were collected from the southern Barents Sea to the marginal ice zone (MIZ) of the Barents Sea during a cruise (ALV-3) on the R/V Jan Mayen in June/July 1999. A series of transect stations (19) were sampled at regular intervals across the Barents Sea (Fig. 1). Additionally, five stations of 24-h duration (Fig. 1) were sampled beginning in 80% ice cover on July 1 (78° 13.67N, 34° 23.02E) and ending in the central Barents Sea on July 9 (73° 47.99N, 31° 44.10E). Water samples were collected from 10-l Niskin bottles just below the

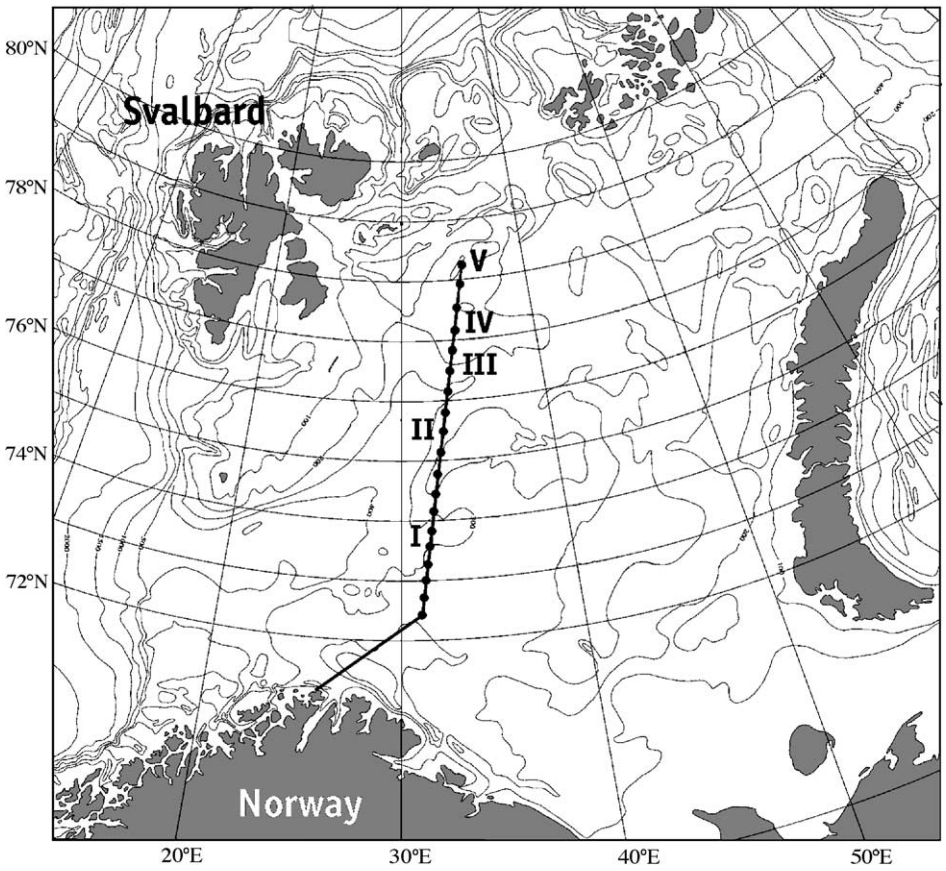


Fig. 1. A map of the cruise transect taken by the R/V Jan Mayen in June 1999. The roman numerals represent the five 24-h stations, with I near the coast and V in the ice. The transect stations fell along the same line as the 24-h stations, with station 1 being the southern most point and station 19 at the northernmost point (ice), with 18 stations in between, each station approximately 20 km apart.

surface (transect) or at various depths (2–200 m) for the 24-h stations. Stations I and II were open ocean stations and sampled at 2, 20, 40, 90 and 200 m. Stations III, IV and V were ice covered stations and were sampled at 2, 20, 40, 90 and 150 m. mVSP water samples (15 ml) were pipetted into 20-ml sterile scintillation vials that contained 5 ml of 99% glycerol (final concentration = 25%), while the remaining sample volume was used for the analyses listed below. All samples were stored in the dark at 4 °C until analyzed.

2.2. 16S rRNA oligonucleotide probes

Cell activity, based on the intracellular concentration of ribosomes, was inferred by hybridization with 16S rRNA-targeted oligonucleotide probes specific for Eubacteria (Braun-Howland et al., 1992). The three oligonucleotides used in this study were Primer A (5′-gwattaccgcggckgctg) (Lane et al., 1985), Primer C (5′-acgggcggtgtgtrc) (Lane et al., 1985) and EUB 342 (5′-ctgctgcsycccgtag) (Vescio and Nierzwicki-Bauer, 1995). Each of the oligonucleotides were modified with a 5′ amino terminus and linked with the fluorochrome FITC, as previously described (Frischer et al., 1996; Williams et al., 1998; Howard-Jones et al., 2001). These probe sets have been used extensively and have been tested for their applicability to microbial investigations (Vescio and Nierzwicki-Bauer, 1995; Frischer et al., 1996; Williams et al., 1998). For the oligonucleotides used in this study, the estimated threshold per cell concentration of RNA required for detection of a cell by

FISH was 0.12 fg RNA/cell (Frischer et al., submitted for publication). This estimate is for the oligonucleotide probe set labeled with TRITC, rather than FITC.

The mVSP protocol employs DAPI as an indicator of total cell numbers. DAPI is a general stain that binds to DNA in all cells regardless of its physiological state. Propidium iodide (PI) is a nucleic acid stain that accumulates in cells with compromised membranes and is excluded from cells with intact membranes. Results of previous work demonstrate that under optimized conditions, PI is an effective vital stain for determining the integrity of bacteria cell membranes (Humphreys et al., 1994; Williams et al., 1998; Howard-Jones et al., 2001). Cells that accumulate and are visualized with PI are considered dead. DAPI and PI were purchased from Sigma (St. Louis, MO, USA). Stock solutions of DAPI (50 μg ml^{-1}) and PI (20 μg ml^{-1}) were prepared in sterile, filtered (Millipore 0.2 μm) distilled water. Stock solutions were stored in the dark at 4 °C until use.

The mVSP protocol equilibrates water samples in 25% glycerol (final concentration) which allows them to be stored at −20 °C. Previous studies have demonstrated that samples preserved in glycerol can be stored for up to 3 months in the freezer (Williams et al., 1998). The mVSP protocol sequentially fixes, stains, permeabilizes and hybridizes cells with 16S rRNA oligonucleotide probes. One to two milliliters of cells (depending on concentration) were fixed with methanol (final concentration = 2%) and incubated at room temperature for 30 min. Cells were then stained with DAPI (60 μl ml^{-1}; final concentration 3 μg ml^{-1}) and PI (10 μl ml^{-1}; final concentration 0.2 μg ml^{-1}) for 30 min at room temperature in the dark. The samples were permeabilized and dehydrated with 200 μl of ethanol/formaldehyde (90:10 v/v) for 30 min, also in the dark. There is no wash-step involved in the staining protocol. The bacterial samples were filtered through 0.2-μm black polycarbonate filters, underlaid with a 25-mm GF/F filter and washed three times with 1-ml aliquots of 10 mM $MgSO_4$ (pH 6.5). The filters were overlaid with the probe mixture in hybridization solution [0.2% w/v bovine serum albumin (BSA), 0.01% w/v polyadenylic acid, 1 × SET (150 mM NaCl, 20 mM Tris–HCl, 1 mM EDTA, pH = 8.0) and 11% dextran sulfate]. The probe mixture was composed of 340 ng of three oligonucleotides (Primer A, C, EUB 342) labeled with FITC. Following hybridization, slides were washed for 30 min in three changes of 1 × SET, air-dried and mounted with low fluorescence immersion oil. For further details, see Williams et al. (1998) and Howard-Jones et al. (2001).

Total cells (DAPI), dead cells (PI) and active cells (probe) of bacteria were enumerated using Wide UV (Exciter filter BP 330–385, dichroic mirror DM 400 and barrier filter BA 420), Wide Green (Excitation filter 510–550, dichroic mirror DM 570 and barrier filter BA 590) and Narrow Blue (Excitation filter BP 470–490, dichroic mirror DM 500 and barrier filter BA 515) filter sets, respectively. The percent of low-activity cells was determined by counting individual cells in all three fields. Low-activity cells are those that were visualized solely in the DAPI field. An Olympus BX-60 fluorescence microscope equipped with a 100× UPLANFL NA 1.35 oil objective was used. The imaging system used here is similar to that described previously (Verity and Sieracki, 1993; Shopov et al., 2000), with upgrades including a Photonics Science cooled three-chip color integrating CCD camera and customized Image ProPlus version 4.0 for Windows (Shopov et al., 2000).

2.3. Viral counts

Viruses were enumerated using epifluorescence microscopy following staining with Yo-Pro (Hennes and Suttle, 1995). Briefly, a stock solution of Yo-Pro (Molecular Probes; 1 mM Yo-Pro in a 1:4 solution of dimethyl sulfoxide and water) was diluted to 50 μM in a solution of 2 mM NaCN. Immediately following water collection, 1–5 ml of unfixed samples containing viruses were gently filtered through a glass tower/manifold set-up onto a 0.02-μm pore size Anodisc filter (Whatman). A series of drops (80 μl) of Yo-Pro solution were dispensed into a 10-cm plastic Petri dish, in the lid of which a piece of filter paper soaked with 3% NaCl solution was placed to prevent evaporation. While still wet, the filters were placed cell side up onto one of the drops and incubated for 2 days in the dark at room temperature. The filters were then washed two times with 1 ml of distilled water, transferred to glass slides and immediately covered with a drop of spectrophotometry grade immersion oil and coverslips. Slides were stored at −20 °C until processed. For each sample, >200 viral particles in 20 randomly selected fields were counted with an Olympus BX-

60 fluorescence microscope equipped with a 100× UPLANFL NA 1.35 oil objective and Narrow Blue (Excitation filter BP 470–490, dichroic mirror DM 500 and barrier filter BA 515) filter set.

2.4. Incorporation of ^{3}[H]-leucine

Rates of incorporation were determined by modifications of the microcentrifugation method for ^{3}H-leucine (Smith and Azam, 1992; Sherr et al., 1999a). Triplicate water samples were incubated with leucine (final concentration 20 nM) in a flow through incubator on board the ship from 1 to 3 h at in situ temperatures, depending on depth, temperature and anticipated activity. For each sample, 1.7-ml aliquots were pipetted into 2-ml microcentrifuge tubes. A fourth sample, which served as a control for abiotic uptake, was killed with 50% (final concentration) trichloroacetic acid (TCA). After incubation, leucine incorporation was terminated by the addition of 190 μl of 50% TCA to each of the live replicates. Samples were extracted and washed in 5% TCA and 80% ethanol and further processed as described by Smith and Azam (1992). Samples were counted in Ecoscint scintillation cocktail.

Killed controls were subtracted from the average of triplicate live DPM values and molar incorporation rates were determined. Incorporation rates of leucine were converted to bacterial production assuming 1.15×10^{17} cells mol^{-1}, which is the mean of all open oceanic studies (Ducklow and Carlson, 1992; Kirchman, 1992). Bacterial density was assumed to be 15 fg C cell^{-1} (Fagerbakke et al., 1996). These two assumptions lead to a 1.725 kg C mol^{-1} conversion factor, which results from assuming no isotope dilution, 7.3% leucine in protein, and that protein is 61.8% of total cellular carbon (Simon and Azam, 1989). Prior work in cold-water environments (Fagerbakke et al., 1996; Carlson et al., 1999) has determined that the more commonly used 3.1 kg C mol^{-1} conversion factor (Simon and Azam, 1989) may overestimate production in these waters. Our conversions of leucine incorporation rates lead to lower production rates and therefore are likely to provide conservative estimates of heterotrophic bacteria productivity.

2.5. Bacterial biomass and growth rate

Bacterial carbon biomass was estimated assuming that one bacterial cell contains 15 fg of carbon (Fagerbakke et al., 1996). Bacterial production rates (mg C m^{-3} day^{-1}) were normalized to bacterial biomass (mg C m^{-3}) in order to calculate average

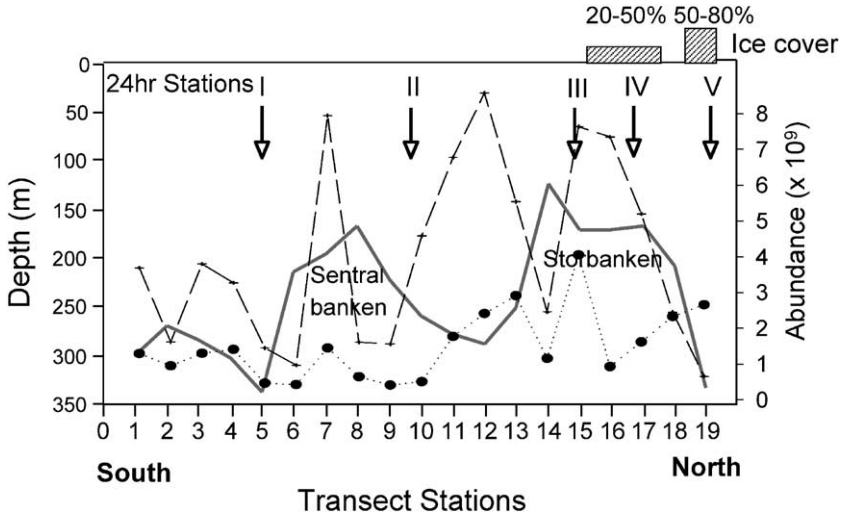


Fig. 2. Bacterial (●) and viral (–) abundance presented as cells or VLP per liter across the Barents Sea. Stations 1–10 are the southern and central Barents Sea, stations 11–14 in the Polar Front and stations 15–19 are with ice cover. Bacterial abundance was determined by DAPI staining; viral abundance with Yo-Pro. Error bars are standard deviations, $n=3$. The solid line represents the bathymetry across the transect.

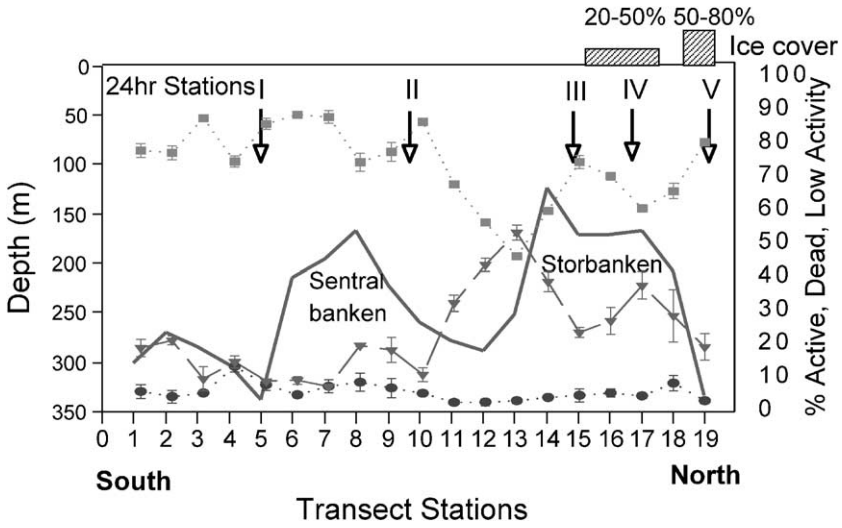


Fig. 3. The proportion of dead (●), active (▼) and low activity (■) bacterioplankton presented as cells per liter across the transect, stations 1–19. Physiological state was determined with the mVSP protocol. Error bars are standard deviations, $n=3$.

growth rates for the bacterioplankton populations in the surface waters and those collected at depth. The turnover or doubling time (h) of the population was determined by the following equation: $(\ln 2)/\mu$, where μ = growth rate (LeBaron et al., 1999).

2.6. Statistical analysis

The data were analyzed by analysis of variance and regression models with SigmaPlot 2000 (version 6.0) and SigmaStat 2.0 (SPSS, Chicago, IL).

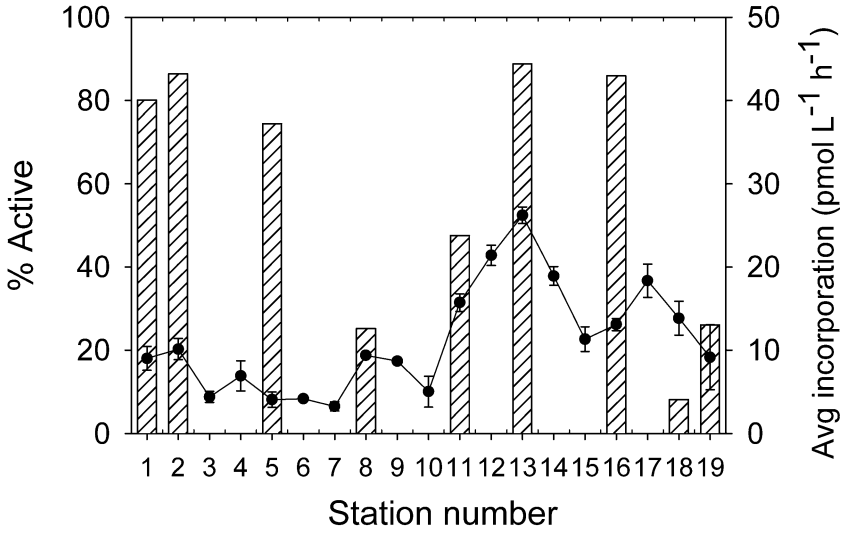


Fig. 4. The relationship between single-cell activity determined by oligonucleotide probes (line) and community activity by leucine incorporation (bars). Activity is plotted by station number. Error bars are standard deviations, $n=3$.

3. Results

3.1. Transect across the Barents Sea

The abundance of bacteria varied from 0.41×10^9 to 4.1×10^9 cells l^{-1} and viruses from 0.66×10^9 to 8.6×10^9 viruses l^{-1} in all samples (Fig. 2). Although there was significant variability, bacterial abundance was generally higher (ANOVA, $p < 0.001$) in the Polar Front (stations 11–14) and Ice drift (stations 15–19) (2.2×10^9 cells l^{-1}) as compared with the Norwegian coastal current and North Atlantic stations (average 0.88×10^9 cells l^{-1}). The highest bacterial abundance was found in the drift ice (station 15), while virus-like particles (VLP) were most numerous in the Polar Front waters (station 12, 8.6×10^9 viruses l^{-1}) (Fig. 2). Viruses displayed no obvious trend, but followed bacterial abundances with increases in the northern stations (after station 10), specifically the Polar Front and ice drift waters. On average, the virus to bacterial cell ratio across the transect was 3:1.

Bacterioplankton in the Polar Front and Marginal Ice Zone (MIZ) contained the largest fraction of active cells as determined by FISH. In general, the fraction of the total cells (DAPI) that contained a sufficient amount of rRNA to be considered active increased significantly from warmer coastal regions (stations 1–7, average 13%) to the colder Arctic waters (stations 8–19, average 27%) ($p < 0.05$), while the proportion of cells that were dead remained relatively constant (average 5%) and were not significantly different (ANOVA, $p > 0.1$) across the transect (Fig. 3). The percent of cells that neither stained with PI (dead) nor hybridized with oligonucleotide probes (active) made up a significant fraction of the cells over the entire transect (45–88%). This group of cells contained DNA (stained with DAPI) and was therefore considered live, but expressed low activity. Alternatively, some fraction of these low-activity cells may be bacteria that are members of the domain Archae.

Leucine incorporation rates for bacterioplankton ranged from <5 to 45 pmol l^{-1} h^{-1} and did not vary with distance from ice coverage. The lowest rate of leucine incorporation (4 pmol l^{-1} h^{-1}) was at station 18, a station that was covered with ice, while the highest incorporation rate (44 pmol l^{-1} h^{-1}) was at station 13, the Polar Front (Fig. 4). The mean incorporation rates for the entire transect was 29 pmol l^{-1} h^{-1}. Across the transect, no correlation existed between activity as estimated with 16S rRNA oligonucleotide probes and community activity as esti-

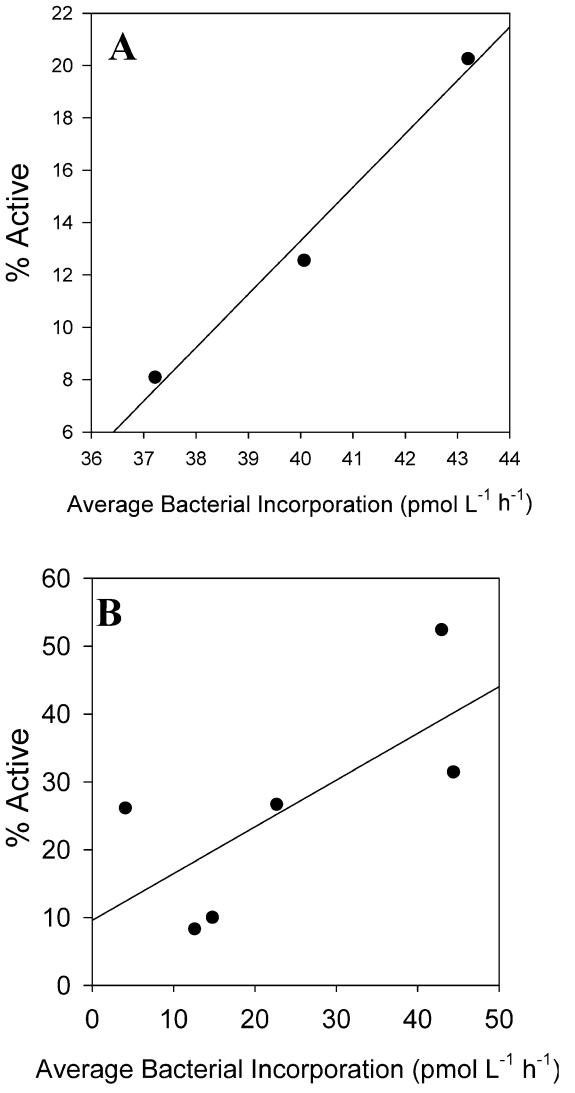


Fig. 5. Results of regression analysis with leucine incorporation as the dependent variable and probes as the independent variable. These regressions are for samples taken along the transect (station 1–19). If the data are separated into similar water masses, the two activity measurements are weakly correlated. The correlation coefficient (r) is given in each panel. (A) Southern Barents Sea (stations 1–7), $r^2 = 0.98$, $n = 3$. (B) Polar Front and ice covered (stations 8–19), $r^2 = 0.62$, $n = 5$.

mated by leucine incorporation. However, if both of the activity measurements were compared in similar masses of water, a better correlation between the two parameters was observed. Station 1–7 represent the "warmer waters" of the Norwegian coastal current and North Atlantic waters with an average surface water temperature of 6.8 °C while stations 8–19 are composed of Polar Front waters and ice drift with an average surface temperature of 1.3 °C (data not shown). Activity was compared in the warmer bodies of water (stations 1–7, Fig. 5A) and in the colder polar waters (Polar Front and ice drift, stations 8–19, Fig. 5B). Good correlations were found between leucine incorporation and 16S rRNA oligonucleotide probes with type II regressions ($r^2 = 0.98$, $n = 3$; $r^2 = 0.6$, $n = 6$).

Bacterial biomass was determined by assuming each cell contained 15 fg C. Community biomass (mg C m^{-3}) was relatively similar among the open ocean stations (average 20.36 mg C m^{-3}), but was significantly higher at stations with >50% ice cover (stations 18–19, average 33.01 mg C m^{-3}) (ANOVA, $p < 0.05$).

Bacterial production rates were estimated in near-surface waters from leucine incorporation rates and assuming a conversion factor of 1.725 kg C mol^{-1}. The maximum production along the transect was 1.8 mg C m^{-3} day^{-1} (station 13, Polar Front), and occurred between two regions of elevated biomass; a secondary maxima in production (average 1.6 mg C m^{-3} day^{-1}) occurred in the coastal front (station 2), a station that did not correspond to elevated bacterial biomass. The highest rates of bacterial production occurred in open waters (station 1–14) and were on average 35 times higher than ice-covered stations (station 15–19). The average production for the nineteen stations was 1.0 mg C m^{-3} day^{-1}.

Bacterial production rates (mg C m^{-3} day^{-1}) and biomass (mg C m^{-3}) were compared to calculate growth rates (production/biomass). Rates for near-surface water samples taken across the transect varied from <0.05 to 0.1 day^{-1}, with an average of 0.06 day^{-1}. The maxima in growth rates overlapped with bacterial productivity maxima (stations 2 and 13). Rates for ice-covered stations were each 8–10 times lower than for open ocean stations (stations 1–14). These growth rates correspond to generation times of 6 to >200 days.

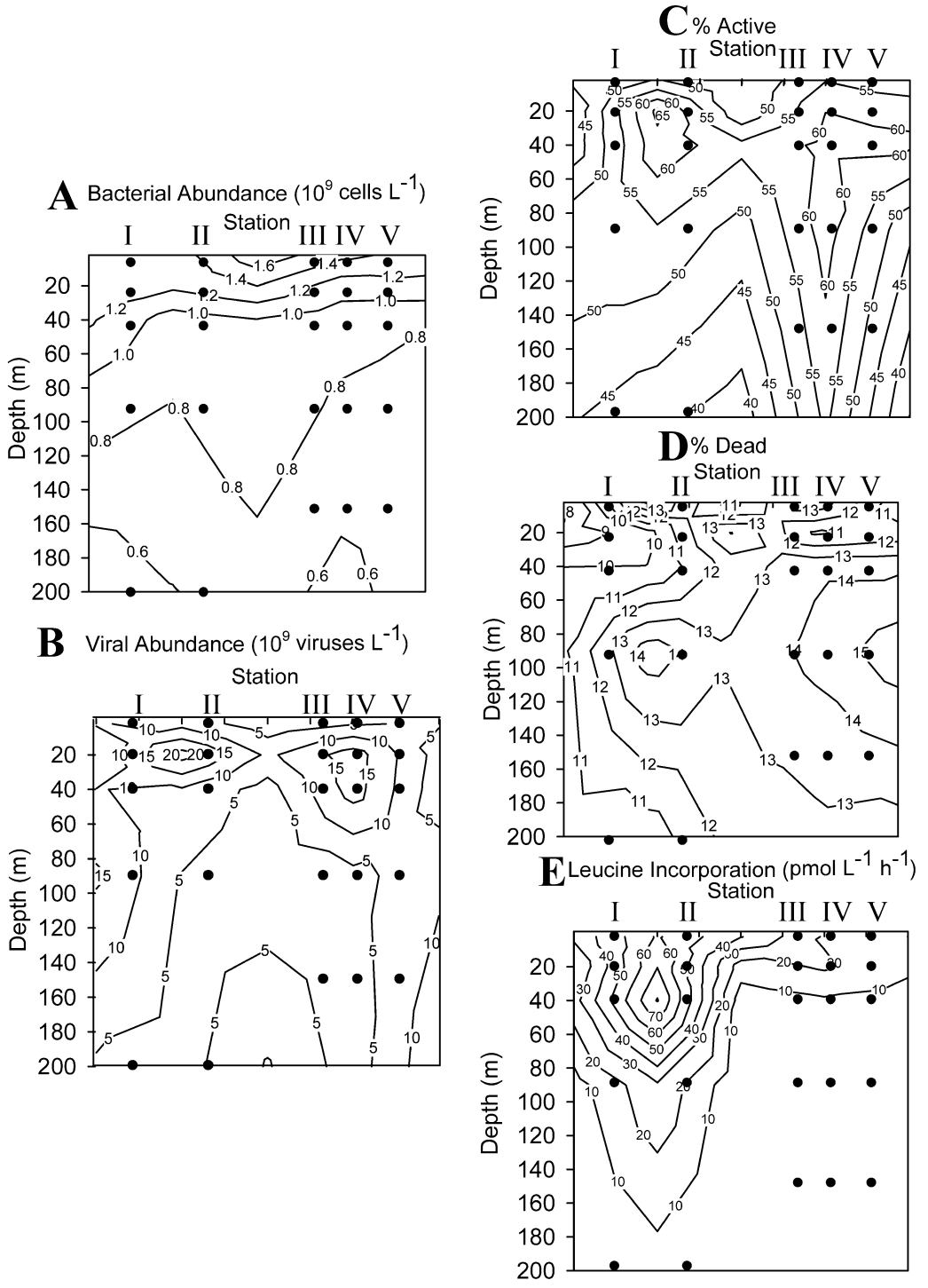
3.2. 24-h stations

For the five 24-h stations, bacterial and viral abundances were determined over depth profiles of 2–200 m (Fig. 6A and B). In general, at all five stations, the total number of bacterioplankton decreased from 1.4×10^9 to 0.59×10^9 cells l^{-1} with increasing depth (Fig. 6A). There was more variability in the abundance of viruses with depth, compared with bacteria. In near-surface waters, the total virus-like particles ranged from 1.9×10^9 to 22×10^9 viruses l^{-1} while at depth, the total number of viruses ranged from 4.2×10^9 to 14×10^9 viruses l^{-1}. Bacteria were most abundant in the near-surface (2 m) while viruses displayed a subsurface peak for all five stations at 20 m.

The average proportion of active cells at the five 24-h stations decreased from surface waters (49%) to depth (42%) (ANOVA, $p < 0.05$) (Fig. 6C). The ice covered waters (III–V), all exhibited a subsurface maximum of active cells at 50 m, just below the chl *a* maximum at 40 m (data not shown) (Reigstad et al., 2002). The open ocean stations (I, II) displayed subsurface maximum of active cells at 30 m (Fig. 6C). The proportions of dead cells in the near surface waters (2 m) and at depth (150–200 m) were statistically similar (ANOVA, $p > 0.1$) (Fig. 6D). Low-activity cells represented 32–46% of the total bacterioplankton population at any given station and depth.

Leucine incorporation rates for bacterioplankton were also determined at the five 24-h stations (Fig. 6E). The mean incorporation rates were consistently higher in the surface waters (2 m, 29 pmol l^{-1} h^{-1}) than at depth (90–200 m, 4.1 pmol l^{-1} h^{-1}), but did not necessarily decrease with depth at each station. There was some variability in leucine incorporation

Fig. 6. Vertical sections of microbial parameters. Points represent the location of the stations and depth of sampling. (A) Bacterial cell abundance determined by DAPI staining. (B) Viruses and VLP determined by Yo-Pro staining. (C) Percent of cells that are considered dead due to compromised membranes. (D) Percent of active bacterial cells determined by hybridization with 16S rRNA oligonucleotide probes. (E) Community activity determined by leucine incorporation.


A Bacterial Abundance (10^9 cells L^{-1})
B Viral Abundance (10^9 viruses L^{-1})
C % Active
D % Dead
E Leucine Incorporation (pmol L^{-1} h^{-1})
Station
I II III IV V
Depth (m)
20 40 60 80 100 120 140 160 180 200

rates in the upper 40 m (stations I–V), then rates remained constant from 90 m and showed little variation between stations below this depth (average 1.7 pmol l^{-1} h^{-1}). Surface samples at the 24-h stations correspond to samples taken as part of the transect (1–19) and average incorporation rates for the five 24-h stations were comparable to the average across the transect (29 and 24 pmol l^{-1} h^{-1}, respectively). The incorporation of radiolabeled leucine was significantly higher at station II (average of depths 2–200 m: 49 pmol l^{-1} h^{-1}; ANOVA, $p<0.001$), an open ocean station, while incorporation was lowest at Station V (average of depths 2–150 m: 9 pmol l^{-1} h^{-1}) where the ice cover was the greatest. The results of direct comparisons between leucine and probes at individual stations were variable. At stations II and V, there was a weak correlation (type II regressions, correlation coefficient $r^2=0.69$ and 0.57, respectively, $n=5$). At stations I, III and IV, no correlation existed ($r^2<0.3$, $n=5$). When activity determined by incor-

Table 1
Summary of parameters measured for each depth sampled at 5–24-h stations along a transect from the coast of Norway to the ice of the Barents Sea. Activity was determined by hybridization with oligonucleotide probes, while the percent dead was determined by staining with PI. Production was calculated from leucine incorporation rates and biomass from DAPI cell counts. Also presented are the growth rates and turnover times, which were determined for each depth at the five stations

Station (distance in m)	Percent active	Percent dead	Leucine incorporation (pmol l^{-1} h^{-1})	Bacterial production (mg C m^{-3} day^{-1})	Biomass (mg C m^{-3})	Growth rate (day^{-1})	Turnover (days)
24 I							
2	51	10	32.2	55.6	20.9	0.064	10.9
20	59	11	19.9	34.3	20.6	0.040	17.4
40	61	14	25.9	44.6	18.4	0.058	11.9
90	46	16	5.7	9.8	13.2	0.018	39.0
200	35	12	1.5	2.6	6.7	0.009	75.9
24 II							
2	55	14	61.3	105.8	19.1	0.133	5.2
20	60	11	69.7	120.2	18.7	0.155	4.5
40	61	14	80.8	139.3	13.4	0.249	2.8
90	62	14	28.6	49.3	11.9	0.099	7.0
200	56	13	5.0	8.6	8.9	0.023	29.7
24 III							
2	45	11	40.6	70.0	26.1	0.064	10.8
20	46	14	14.9	25.6	21.0	0.029	23.6
40	56	12	4.7	8.1	14.9	0.013	52.7
90	48	13	1.3	2.2	14.2	0.004	183.8
150	37	12	1.9	3.2	10.5	0.007	93.5
24 IV							
2	50	14	19.0	32.7	21.8	0.036	19.2
20	66	10	21.4	36.9	16.4	0.054	12.8
40	63	10	8.7	15.0	13.3	0.027	25.7
90	54	15	0.9	1.5	11.4	0.003	216.7
150	41	10	0.6	1.0	8.0	0.003	237.8
24 V							
2	46	7	9.9	17.1	19.8	0.021	33.4
20	41	8	11.7	20.3	17.0	0.029	24.3
40	42	10	6.4	11.0	12.2	0.022	31.9
90	54	11	0.7	1.2	10.7	0.003	249.5
150	45	11	0.5	0.8	10.7	0.002	376.0

poration and probes were integrated across the Barents Sea, no apparent correlation existed ($r^2 = 0.01$, $n = 25$).

The average bacterial biomass for surface samples (2 m) was 21.5 mg C m^{-3}. Community biomass (mg C m^{-3}) decreased with depth to an average 9.0 mg C m^{-3} at 150/200 m. Unlike the transect stations, the bacterial biomass was similar among the five stations at the surface and with depth (ANOVA, $p>0.1$) (Table 1). The average bacterial biomass for the transect (2 m surface average, 29.3 mg C m^{-3}) was higher in comparison with the 24-h stations (2 m surface average, 21.5 mg C m^{-3}).

Bacterial production rates revealed a maximum in surface waters (2 m) at the 24-h stations (1.35 mg C m^{-3} day^{-1}), with a gradual decrease to 0.08 mg C m^{-3} day^{-1} at depth (150/200 m). Bacterial production was consistently higher at station II, an open ocean station with rates up to 10 times higher in surface waters and at depth, in comparison to the other four stations (Table 1). On average, the open ocean stations (I, II) exhibited higher production rates (1.94 mg C m^{-3} day^{-1}, 2 m; 0.04 mg C m^{-3} day^{-1}, 150 m).

Near-surface waters (0–50 m) at the 24-h stations exhibited growth rates of bacterioplankton ranging from 0.02 to 0.24 day^{-1} with the higher values corresponding to depths with bacterial productivity maxima (station II). The average growth rate in surface waters (2 m) for the 24-h stations was 0.06 day^{-1}, matching the rate obtained for the transect. These growth rates correspond to generation times of 2 to >200 days.

4. Discussion

This study assesses primarily the variability in abundance and metabolic activity of bacteria in the Barents Sea. The question of whether bacterioplankton are predominantly composed of active, dead or low-activity cells has important implications for the understanding of bacteria-mediated processes. For example, low-activity or dormant cells are an ecologically significant proportion of the community than can potentially respond to nutrient pulses (Allen et al., 2002). In this study, activity was determined by assays based on cellular ribosomal content (16S rRNA oligonucleotide probes) and community uptake of radiolabeled compounds (^{3}H-Leucine), while a vital stain was used to assess bacterial cell membrane integrity. This study provides a quantitative estimate of bacterial and viral abundance as well as estimates of bacterial growth rates and activity in Arctic waters.

4.1. Abundance

This data indicates that bacterial abundance increased from the coast of Norway (station 1) to the Polar Front (stations 11–14) and ice stations (stations 15–19). Depth profiles (2–200 m) suggest that bacterioplankton abundance decreased with depth in both coastal and polar waters. The Barents Sea is characterized by subzero temperatures, however the bacterial abundances determined (DAPI counts, average 0.9×10^9 cells l^{-1}), were quite similar to those found in more temperate regions.

Many of the previous investigations of Arctic ecosystems have not included viruses, however our study indicates that they are a ubiquitous and dynamic component of the Arctic marine food web. VLP were abundant in surface seawater samples, and were significantly higher than previously published (Bergh et al., 1989; Bratbak et al., 1990). Previous studies of aquatic ecosystems suggest that viruses and viral lysis influence C flow through the pelagic microbial ecosystem (Bratbak et al., 1992, 1998) by diverting the cells' particulate production into dissolved organic matter (Bratbak et al., 1998).

4.2. Activity

The results from this study demonstrate that at least for the communities studied (Eubacteria), active bacteria were more abundant in colder waters. The higher measurements of activity in these waters may be due to increased stratification and nutrient availability in surface waters resulting from Arctic spring blooms, (Reigstad et al., 2002) which produce biomass accumulation. Increased bacterial activity may also be due to the release of microbial cells from sea ice (Sullivan et al., 1990). Activity measured by probes increased from <20% in the southern Barents Sea (stations 1–6) to peak at >50% in the Polar Front waters and was 38% at the first station with ice (station 15). At the five 24-h stations, the proportion of active cells

displayed a subsurface peak of activity (60%) at 50 m in the ice-covered waters (III–V), just below the chlorophyll maximum at 40 m (Reigstad et al., 2002) and at a depth of 30 m (65%) in the open ocean stations (I and II).

Two activity measurements were examined and compared, 16S rRNA oligonucleotide probes and leucine incorporation. Leucine incorporation rates ranged from <5 to 45 pmol l^{-1} h^{-1}, similar to data previously published for Arctic bacterioplankton (Steward et al., 1996; Sherr et al., 1997). Leucine incorporation rates were highest in Polar Front waters (station 13) and then decreased at stations 15 through 19 (ice covered). For the 24-h stations, leucine incorporation rates were significantly higher in surface waters than at depth, and the highest rates were observed at station II, an open ocean station.

For the first half of the transect, activity determined by probes suggested that very few organisms were active (20–30%), while leucine incorporation implied that the community was very productive (in comparison to other stations). There are several hypotheses that could explain the differences in these two activity measurements including: (1) fewer cells were contributing to the higher community measurement of activity, (2) bacterial cells that occupy Arctic water masses maintain higher cellular rRNA contents relative to their productivity than do coastal bacterioplankton populations, (3) cells found in colder Arctic waters have greater per cell RNA content than cells typically found in warmer waters, or (4) results from plankton measurements revealed a postbloom situation in the southern and central Barents Sea (Verity et al., 2002). The opposite scenario was found at the northern end of the transect (including Polar Front and ice covered stations), e.g. at stations 11–19, probe hybridization estimated that 35–50% of the total population was metabolically active, while leucine incorporation rates were <1 pmol l^{-1} h^{-1}. Data at these stations suggested that a larger fraction of the community was contributing to a much smaller community productivity. The pattern of activity exhibited by the bacterial community at these northern stations (15–19) might be due to enhanced primary productivity in the MIZ, coupled with colder temperatures (Verity et al., 2002).

Another possible source of error or difference between these two activity measurements is that some fraction of the leucine that is taken up by the cells may not be metabolized and incorporated into protein. The production and growth rates of mixed natural assemblages are diverse. Individual microbial cells express varying growth strategies and therefore can be limited by numerous different factors including: inorganic nutrients, DOM (C, N, P-limited), DFAA, DOM quality or DOM supply (Kirchman, 1990). Bacteria may take up leucine and not incorporate it into proteins directly but rather scavenge the carbon or use the compound for other steady state processes, thereby overestimating the production of the community.

A lack of correlation between these activity measurements is not unexpected since the two methods target different physiological characteristics of bacterioplankton. Oligonucleotide probes, which bind ribosomal RNA, were used as indicators of single cell metabolic activity, such that a cell containing a high copy number of ribosomes is showing the potential to be metabolically active. Community activity was assessed by the active uptake and incorporation of amino acids (radiolabeled leucine) into proteins (Karner and Fuhrman, 1997). This study suggests that leucine incorporation and 16S rRNA oligonucleotide probes do not target the same fraction or subpopulation of the community.

Production rates were estimated for bacterioplankton populations from leucine incorporation. Rates decreased with depth, but remained in the range of those previously reported for Arctic populations (0.02–1.5 mg C m^{-3} day^{-1}), some using different techniques (Cota et al., 1996; Steward et al., 1996; Rich et al., 1997). These production rates were also used to estimate heterotrophic bacterial nitrogen demand, and correlated well with N^{15} data (Allen et al., 2002).

4.3. Bacterial biomass and growth rates

Bacterial biomass was similar among open ocean stations (1–14) across the transect but higher in ice covered stations (15–19). Community biomass decreased gradually with depth at all 5–24-h stations. Both activity measurements showed significant fluctuations across the transect and with depth that did not parallel abundance, suggesting that the variability seen in activity was most likely not due to changes

in the total number of cells. Growth rates were highly variable although within the range (<0.05–0.6 day^{-1}) of previously published data (Kottmeier et al., 1987; Cota et al., 1990; Rich et al., 1997) with a maximum of 0.24 day^{-1} (24 II, 60 m) and minimum of 0.0004 day^{-1} (station 18, 2 m), implying generation times of 2 to >200 days, respectively. Growth rates determined for surface waters across the transect and at the 24-h stations were identical, 0.06 day^{-1}.

4.4. Physiological status: dead or low activity

The fraction of dead cells (compromised membranes) remained relatively constant over the transect and made up only a small fraction (<10%) of the total cells, while low-activity cells made up the largest proportion of total cells (45–88%). The fraction of dead cells were statistically similar at the surface and with depth, while low-activity cells were a significant proportion of the total cells at the five 24-h stations (32–46%). When the fraction of low-activity and dead cells are combined, they account for a large fraction of the total cells (40–90%). A general review of the literature supports this finding and suggests that a significant fraction of bacterioplankton cells in the marine environment are dead or relatively inactive (Del Giorgio and Scarborough, 1995; Zweifel and Hagstrom, 1995; Sherr et al., 1999b). The question of whether or not these cells are growing, dividing, or even viable is crucial for our understanding of their potential role in these ecosystems. It is our hypothesis that this fraction consists of cells of various physiological states including cells that are undergoing division, viral infection, cells that are living yet not growing, and possibly a fraction of these cells may be bacterioplankton that are members of the domain Archaea (DeLong et al., 1994; Fuhrman and Ouverney, 1998).

Bacterial abundance and activities (probes and leucine) measured in the Barents Sea are similar to those for low latitude open ocean waters, where bacteria and the microbial loop are well accepted as critical components of food webs (Pomeroy, 1974; Fuhrman and Azam, 1980; Hodson et al., 1981 Hanson and Lowery, 1983; Cho and Azam, 1988; Ducklow and Carlson, 1992; Rivkin et al., 1991). This study demonstrates the potential productivity of the Arctic bacterioplankton and show that they are far more important in Arctic food webs than previously assumed (Gran, 1902; Ussing, 1938; Smidt, 1979).

Several conclusions about the physiological status of Arctic bacterioplankton emerge: (1) a small fraction of the total bacterial cells are dead, (2) a significant fraction (25–80%) of the total bacteria are dormant or express very low activity, and (3) the fraction of active bacteria varies depending on the method used. The question arises as to how the activity measurements should be interpreted. One hypothesis is that aquatic microbial communities are composed of dynamic populations, capable of responding to local changes in the physical and chemical environment. Understanding the dominant role cold-water ecosystems have in biogeochemical pathways can only be improved if the identity, activity, and function of individual microbial cells in these environments are determined.

Acknowledgements

We gratefully acknowledge S. Øygarden, C. Wexel-Riser and M. Reigstad for their assistance in preparing for the cruise and the countless hours they spent assuring that we had the necessary supplies and resources before the cruise departed. We would also like to thank Paul Wassmann for his continuous support and the invitation to participate on this research cruise. We are grateful to the captain and the crew of R/V Jay Mayen for their support and assistance during the entire cruise. This research was supported by NSF grants OCE-95-21086 and 99-82133 and DOE grant FG-02-98ER62531.

References

Allen, A.E., Howard-Jones, M.H., Booth, M.G., Frischer, M.E., Verity, P.G., Bronk, D.A., Sanderson, M.P., 2002. Importance of heterotrophic bacterial assimilation of ammonium and nitrate in the Barents Sea during summer. J. Mar. Syst. 38, 93–108 (this volume).

Azam, F., Fenchel, T., Field, J.G., Gray, J.S., Meyer-Reil, L.A., Thingstad, F., Meyer-Reil, L.A., 1983. The ecological role of water column microbes in the sea. Mar. Ecol. Prog. Ser. 10, 257–263.

Bergh, O., Børsheim, K.Y., Bratbak, G., Heldal, M., 1989. High abundance of viruses found in aquatic environments. Nature 340, 467–468.

Bratbak, G., Heldal, M., Norland, S., Thingstad, T.F., 1990. Viruses

as partners in spring bloom microbial trophodynamics. Appl. Environ. Microbiol. 56, 1400–1405.

Bratbak, G., Heldal, M., Thingstad, T.F., Riemann, B., Haslund, O.H., 1992. Incorporation of viruses into the budget of microbial C-transfer. A first approach. Mar. Ecol. Prog. Ser 83, 273–280.

Bratbak, G., Jacobsen, A., Heldal, M., 1998. Viral lysis of *Phaeocystis pouchetii* and bacterial secondary production. Aquat. Microb. Ecol. 16, 11–16.

Braun-Howland, E.B., Danielsen, S., Nierzwicki-Bauer, S.A., 1992. Development of a rapid method for detecting bacterial cells in situ using 16S rRNA-targeted probes. Biotechniques 13, 928–934.

Carlson, C.A., Bates, N.R., Ducklow, H.W., Hansell, D.A., 1999. Estimation of bacterial respiration and growth efficiency in the Ross Sea, Antarctica. Aquat. Microb. Ecol. 19, 229–244.

Cho, B.C., Azam, F., 1988. Major role of bacteria in biogeochemical fluxes in the ocean's interior. Nature 332, 441–443.

Cota, G.F., Kottmeier, S.T., Robinson, D.H., Smith, W.O., Sullivan, C.W., 1990. Bacterioplankton in the marginal ice zone of the Weddell Sea: biomass, production and metabolic activities during austral autumn. Deep-Sea Res. I 37, 1145–1167.

Cota, G.F., Pomeroy, L.R., Harrison, W.G., Jones, E.P., Peters, F., Sheldon, W.M., Weingartner, T.R., 1996. Nutrients, primary productivity, and microbial heterotrophy in the southeast Chukchi Sea: Arctic summer nutrient depletion and heterotrophy. Mar. Ecol. Prog. Ser. 135, 247–258.

Del Giorgio, P.A., Scarborough, G., 1995. Increase in the proportion of metabolically active bacteria along gradients of enrichment in freshwater and marine plankton: implications for estimates of bacterial growth and production. J. Plankton Res. 17, 1905–1924.

DeLong, E.F., Wu, K.Y., Prezelin, B.B., Jovine, R.V.M., 1994. High abundance of Archae in Antarctic marine picoplankton. Nature 371, 695–697.

Ducklow, H.W., Carlson, C.A., 1992. Oceanic bacterial production. Adv. Microb. Ecol. 12, 113–181.

Fagerbakke, K.M., Heldal, M., Norland, S., 1996. Content of carbon, nitrogen, sulfur, and phosphorus in native aquatic and cultured bacteria. Aquat. Microb. Ecol. 10, 15–27.

Frischer, M.E., Floriani, P.J., Nierzwicki-Bauer, S.A., 1996. Differential sensitivity of 16S rRNA targeted oligonucleotide probes for fluorescence in situ hybridization is a result of ribosomal higher order structure. Can. J. Microbiol. 42, 1061–1071.

Frischer, M.E., Hong, Y., Howard-Jones, M.H., Verity, P.G., submitted for publication. In situ ribosomal RNA degradation in cultured and natural marine bacteria: VSP identification of dead but recently active cells. Aquat. Microb. Ecol.

Fuhrman, J.A., Azam, F., 1980. Bacterioplankton secondary production estimates for coastal waters of British Columbia, Antarctica and California. Appl. Environ. Microbiol. 39, 1085–1095.

Fuhrman, J.A., Ouverney, C.C., 1998. Marine microbial diversity studied via 16S rRNA sequences: cloning results from coastal waters and counting of native Archae with fluorescent single cell probes. Aquat. Ecol. 32, 3–15.

Fuhrman, J.A., Suttle, C.A., 1993. Viruses in marine planktonic systems. Oceanography 6, 51–63.

Gran, H.H., 1902. Das Plankton des Norwegischen Nordmeeres. Report on Norwegian Fishery- and Marine-investigations, vol. II(5), 222.

Hanson, R.B., Lowery, K.H., 1983. Nucleic acid synthesis in oceanic microplankton from Drakes Passage, Antarctica: evaluation of steady-state growth. Mar. Biol. 73, 79–89.

Hennes, K.P., Suttle, C.A., 1995. Direct counts of viruses in natural waters and laboratory cultures by epifluorescence microscopy. Limnol. Oceanogr. 40, 1050–1055.

Hobbie, J.E., Daley, R.J., Jasper, S., 1977. Use of nucleopore filters for counting bacteria by fluorescence microscopy. Appl. Environ. Microbiol. 33, 1225–1228.

Hodson, R.E., Azam, F., Carlucci, A.F., Fuhrman, J.A., Karl, D.M., Holm-Hansen, O., 1981. Microbial uptake of dissolved organic matter in McMurdo Sound, Antarctica. Mar. Biol. 61, 89–94.

Howard-Jones, M.H., Frischer, M.E., Verity, P.G., 2001. Determining the physiological status of individual bacteria cells. In: Paul, J.H. (Ed.), Methods in Microbiology. Marine Microbiology, vol. 30. Academic Press, London.

Humphreys, M.J., Allman, R., Lloyd, D., 1994. Determination of the viability of *Trichomonas vaginalis* using flow cytometry. Cytometry 15, 343–348.

Karner, M., Fuhrman, J., 1997. Determination of "active" marine bacterioplankton: a comparison of universal 16S rRNA probes, autoradiography, and nucleoid staining. Appl. Environ. Microbiol. 63 (4), 1208–1213.

Kepner Jr., R.L., Pratt, J.R., 1994. Use of fluorochromes for direct enumeration of total bacteria in environmental samples: past and present. Microbiol. Rev. 58, 603–615.

Kirchman, D.L., 1990. Limitation of bacterial growth by dissolved organic matter in the subarctic Pacific. Mar. Ecol. Prog. Ser. 62, 47–54.

Kirchman, D.L., 1992. Incorporation of thymidine and leucine in the subarctic Pacific: application to estimating bacterial production. Mar. Ecol. Prog. Ser. 82, 301–309.

Kirchman, D.L., K'nees, E., Hodson, R.E., 1985. Leucine incorporation and its potential as a measure of protein synthesis and by bacteria in natural aquatic systems. Appl. Environ. Microbiol. 49, 599–607.

Kirchman, D.L., Newell, S.Y., Hodson, R.E., 1986. Incorporation versus biosynthesis of leucine: implications for measuring rates of protein synthesis and biomass production by bacteria in marine systems. Mar. Ecol. Prog. Ser. 32, 47–59.

Kottmeier, S.T., Grossi, S.M., Sullivan, C.W., 1987. Sea ice microbial communities: VIII. Bacterial production in annual sea ice of McMurdo Sound, Antarctica. Mar. Ecol. Prog. Ser. 35, 175–186.

Lane, D.J., Pace, B., Olsen, G.J., Stahl, D.A., Sogin, M.L., Pace, N.R., 1985. Rapid determination of 16S ribosomal RNA sequences for phylogenetic analyses. Proc. Natl. Acad. Sci. U.S.A. 82, 6955–6959.

LeBaron, P., Servais, P., Troussellier, M., Courties, C., Vives-Rego, J., Muyzer, G., Bernard, L., Guindulain, T., Schafer, H., Stackebrandt, E., 1999. Changes in bacterial community structure in seawater mesocosms differing in their nutrient status. Aquat. Microbiol. Ecol. 19, 255–267.

Luchetta, A., Lipizer, M., Socal, G., 2000. Temporal evolution of primary production in the central Barents Sea. J. Mar. Syst. 27, 177–193.

Olsson, K., Andersson, L.G., Frank, M., Luchetta, A., Smethie, W., 1999. Carbon utilization in the Eurasian sector of the Arctic Ocean. Limnol. Oceanogr. 44, 5–105.

Pomeroy, L.R., 1974. The ocean's food web, a changing paradigm. Bioscience 24, 499–504.

Reigstad, M., Wassmann, P., Wexels Riser, C., Øygarden, S., Rey, F., 2002. Variations in hydrography, nutrients and chlorophyll *a* in the marginal ice zone and the central Barents Sea. J. Mar. Syst. 38, 9–29 (this volume).

Rich, J., Gosselin, M., Sherr, E., Sherr, B., Kirchman, D.L., 1997. High bacterial production, uptake, and concentrations of dissolved organic matter in the Central Arctic Ocean. Deep-Sea Res., Part 2 44 (8), 1645–1663.

Rivkin, R.B., Anderson, M.R., Gustafson, D.E., 1991. Dynamics of bacterioplankton growth in McMurdo Sound, Antarctica: evidence for substrate sufficient growth. Antarc. J. U.S. 26, 145–146.

Sambrotto, R.N., Goering, J.J., McRoy, C.P., 1984. Large yearly production of phytoplankton in the Bering Strait. Science 255, 1147–1150.

Sherr, E.B., Sherr, B.F., Fessenden, L., 1997. Heterotrophic protists in the central Arctic Ocean. Deep-Sea Res., Part 2 44, 1665–1682.

Sherr, B.F., del Giorgio, P., Sherr, E.B., 1999a. Estimating abundance and single cell characteristics of actively respiring bacteria via the redox dye, CTC. Aquat. Microb. Ecol. 18, 117–131.

Sherr, E.B., Sherr, B.F., Sigmon, C.T., 1999b. Activity of marine bacteria under incubated and in situ conditions. Aquat. Microbiol. Ecol. 20, 213–223.

Shopov, A., Williams, S.C., Verity, P.G., 2000. Image analysis for the discrimination and enumeration of bacteria and viruses in aquatic samples. Aquat. Microb. Ecol. 22, 103–111.

Simon, M., Azam, F., 1989. Protein content and protein synthesis rates of planktonic marine bacteria. Mar. Ecol. Prog. Ser. 51, 201–213.

Slagstad, D., Wassmann, P., 1997. Climate forcing and carbon flux in the Barents Sea: 3D simulations of ice distribution, primary production, and vertical export of particulate organic matter. Mem. Natl. Inst. Polar Res., Spec. Issue 51, 119–141.

Smidt, E.L.B., 1979. Annual cycles of primary production and of zooplankton at southwest Greenland. Biosci. 1, 1–53.

Smith, D.C., Azam, F., 1992. A simple, economical method for measuring bacterial protein synthesis rates in seawater using ^{3}H-leucine. Mar. Microb. Food Webs 6, 107–114.

Smith, D.C., Steward, G.F., Long, R.A., Azam, F., 1995. Bacterial mediation of carbon fluxes during a diatom bloom in a mesocosm. Deep-Sea Res., Part 2 (42), 75–97.

Steward, G.F., Smith, D.C., Azam, F., 1996. Abundance and production of bacteria and viruses in the Bering and Chukchi Seas. Mar. Ecol. Prog. Ser. 131, 287–300.

Stockner, J.G., Anita, N.J., 1986. Algal picoplankton from marine and freshwater ecosystems: a multidisciplinary perspective. Can. J. Fish. Aquat. Sci. 43, 2472–2503.

Sullivan, C.W., Cota, G.F., Krempin, D.W., Smith, W.O., 1990. Distribution and activity of bacterioplankton in the marginal ice zone of the Weddell–Scotia Sea during austral spring. Mar. Ecol. Prog. Ser. 63, 239–252.

Thingstad, T.F., Martinussen, I., 1991. Are bacteria active in the cold pelagic ecosystem of the Barents Sea? Polar Res. 10, 255–266.

Ussing, H.H., 1938. The biology of some important plankton animals in the fjords of east Greenland. Medd. Grønl. 100 (7), 1–108.

Verity, P.G., Sieracki, M.E., 1993. Use of color image analysis and epifluorescent microscopy to measure plankton biomass. In: Kemp, P.F., Sherr, B.F., Sherr, E.B., Cole, J.J. (Eds.), Handbook of Methods in Aquatic Microbial Ecology. Lewis, New York, pp. 327–338.

Verity, P.G., Wassmann, P., Frischer, M.E., Howard-Jones, M.H., Allen, A.E., 2002. Grazing of phytoplankton by microzooplankton in the Barents Sea during early summer. J. Mar. Syst. 38, 109–123 (this volume).

Vescio, P.A., Nierzwicki-Bauer, S.A., 1995. Extraction and purification of PCR amplifiable DNA from lacustrine subsurface sediments. J. Microbiol. Methods 21, 225–233.

Walsh, J.J., 1989. Arctic carbon sinks: present and future. Glob. Biogeochem. Cycles 3, 393–411.

Williams, S.C., Hong, Y., Danavall, D.C.A., Howard-Jones, M.H., Gibson, D., Frischer, M.E., Verity, P.G., 1998. Distinguishing between living and nonliving bacteria: evaluation of the vital stain propidium iodide and its combined use with molecular probes in aquatic samples. J. Microbiol. Methods 32, 225–236.

Zweifel, U.L., Hagstrom, A., 1995. Total counts of marine bacteria include a large fraction of non-nucleoid-containing bacteria (ghosts). Appl. Environ. Microbiol. 61, 2180–2185.