Volume 54 Number 10 October 2007 ISSN 0967-0637

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DEEP-SEA RESEARCH

PART I

Oceanographic Research Papers

Editor:
Michael P. Bacon
Woods Hole, MA, USA

(Contents continued on outside back cover)

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ScienceDirect
Deep-Sea Research I 54 (2007) 1744–1757
DEEP-SEA RESEARCH PART I
www.elsevier.com/locate/dsri

Microbial biomass and viral infections of heterotrophic prokaryotes in the sub-surface layer of the central Arctic Ocean

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Received 30 September 2006; received in revised form 20 April 2007; accepted 26 April 2007
Available online 16 May 2007

Abstract

Seawater samples were collected for microbial analyses between 55 and 235 m depth across the Arctic Ocean during the SCICEX 97 expedition (03 September–02 October 1997) using a nuclear submarine as a research platform. Abundances of prokaryotes (range 0.043–0.47 $\times 10^9$ dm^{-3}) and viruses (range 0.68–11 $\times 10^9$ dm^{-3}) were correlated ($r = 0.66$, $n = 150$) with an average virus:prokaryote ratio of 26 (range 5–70). Biomass of prokaryotes integrated from 55 to 235 m ranged from 0.27 to 0.85 g C m^{-2} exceeding that of phytoplankton (0.005–0.2 g C m^{-2}) or viruses (0.02–0.05 g C m^{-2}) over the same depth range by an order of magnitude on average. Using transmission electron microscopy (TEM), we estimated that 0.5% of the prokaryote community on average (range 0–1.4%) was visibly infected with viruses, which suggests that very little of prokaryotic secondary production was lost due to viral lysis. Intracellular viruses ranged from 5 to >200/cell, with an average apparent burst size of 45 ± 38 (mean ± s.d.; $n = 45$). TEM also revealed the presence of putative metal-precipitating bacteria in 8 of 13 samples, which averaged 0.3% of the total prokaryote community (range 0–1%). If these prokaryotes are accessible to protistan grazers, the Fe and Mn associated with their capsules might be an important source of trace metals to the planktonic food web. After combining our abundance and mortality data with data from the literature, we conclude that the biomass of prokaryoplankton exceeds that of phytoplankton when averaged over the upper 250 m of the central Arctic Ocean and that the fate of this biomass is poorly understood.

Keywords: Bacteria; Phytoplankton; Viruses; Bacteriophages; Biomass; Mortality

1. Introduction

Prokaryotes are a major conduit for carbon and energy in the ocean. They make up a significant fraction of total biomass (Gasol et al., 1997), and their carbon demand often represents a large fraction of total net primary productivity (Ducklow and Carlson, 1993). Because of their major contributions to marine biogeochemical cycles, the productivity, abundance and distribution of heterotrophic prokaryotes have been intensively studied for most of the world's oceans (Ducklow, 1999), but

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doi:10.1016/j.dsr.2007.04.019

data for the central Arctic Ocean are still few. Results from the Arctic Ocean Section study of 1994 revealed an active microbial food web across this cold, predominantly ice-covered region as far north as the North Pole (Wheeler et al., 1996). Interannual variability appears to be high, however, since a subsequent study in roughly the same area and time of year in 1998 yielded 1.5- to 2-fold lower bacterial abundance (Sherr et al., 2003) and rates of bacterial production an order of magnitude lower (Sherr et al., 2003).

Two major processes removing prokaryotes produced in seawater are consumption by protozoa and lysis by viruses. These two processes are believed to have different effects on the movement of carbon through the food web. Relative to protistan grazing, viral lysis is expected to favor microbial respiration of organic carbon to CO_2 with a concomitant decrease in transfer to higher trophic levels (Fuhrman, 1999). Grazing and viral lysis may be comparable in some cases (Fuhrman and Noble, 1995; Guixa-Boixereu et al., 1999), but many estimates suggest that viral lysis accounts for significantly less than half of the total mortality of prokaryotes (Binder, 1999).

The abundance and distribution of marine viruses and their contributions to planktonic food web dynamics have been intensively studied for over 15 years (Bergh et al., 1989; Suttle, 2005; Wommack and Colwell, 2000), but much of this work has focused on temperate coastal waters. The few reports on viral ecology in Arctic waters suggest that concentrations of viruses at the highest latitudes are about ten times lower than in temperate surface waters (Maranger et al., 1994; Middelboe et al., 2002; Steward et al., 1996; Yager et al., 2001). Estimates of the contribution of viruses to bacterial mortality in Arctic waters have varied with location. Dilution experiments with particle-rich near-bottom water in coastal waters of Baffin Bay seem to indicate that $>60\%$ of bacterial production might be lost to viral lysis (Wells and Deming, 2006). Mortality of prokaryotes attributable to viral infections was reported to be 9–36% in productive coastal waters of the Bering Sea (Steward et al., 1996) and 6–28% in a polynya in Baffin Bay (Middelboe et al., 2002). In contrast, viruses accounted for only about 2–10% of the mortality in the less productive waters of the Chukchi Sea (Steward et al., 1996).

There have not yet been reports on the abundance and impact of viruses in the central Arctic Ocean, but positive correlations with indices of biological productivity (Middelboe et al., 2002; Steward et al., 1996) suggest that both viral abundance and the incidence of viral infections among prokaryotes are likely to be very low in the persistently ice-covered waters to the north. Estimates from one study of grazing by protists in the central Arctic Ocean suggested that this was a significant source of mortality for phytoplankton, but grazing did not account for the majority of bacterial mortality (Sherr et al., 1997). If viral mortality of prokaryotes were also low in the central Arctic Ocean, then the fate of secondary production by prokaryotes in this major ocean basin would remain very poorly constrained.

With models pointing to the Arctic Ocean as an important variable mediating the progression and consequences of global climate change (Steffen, 2006; Walsh, 1989), there is a need to better understand the stocks of organic carbon in this region and the pathways by which carbon is channeled in the food web. In this paper, we present data on the abundance and biomass of phytoplankton, prokaryotes and viruses across the Arctic Ocean Basin between 55 and 235 m depth, a range that encompasses the sub-surface layer of the Arctic Surface Water and the underlying Atlantic Water. We also present the first data on the incidence of viral infections of prokaryotes and the occurrence of putative Fe- and Mn-precipitating prokaryotes in this region.

2. Materials and methods

2.1. Sampling program

Data and samples were collected from aboard a US Navy nuclear submarine, USS *Archerfish*, as part of the SCICEX 97 expedition, which was one of a series of Arctic science expeditions sponsored by the United States National Science Foundation and the Office of Naval Research (Edwards and Coakley, 2003). The sampling for this expedition was conducted from 03 September to 02 October 1997 in the Arctic Ocean primarily under ice cover (Fig. 1). Hydrographic data were collected by three methods. The first was a pair of continuous recording SEACAT conductivity, temperature, and depth recorders and dissolved oxygen sensors (CTD; SeaBird, Inc.) mounted in the ship's sail. The second was periodic deployment of under-ice, submarine-launched, expendable probes measuring

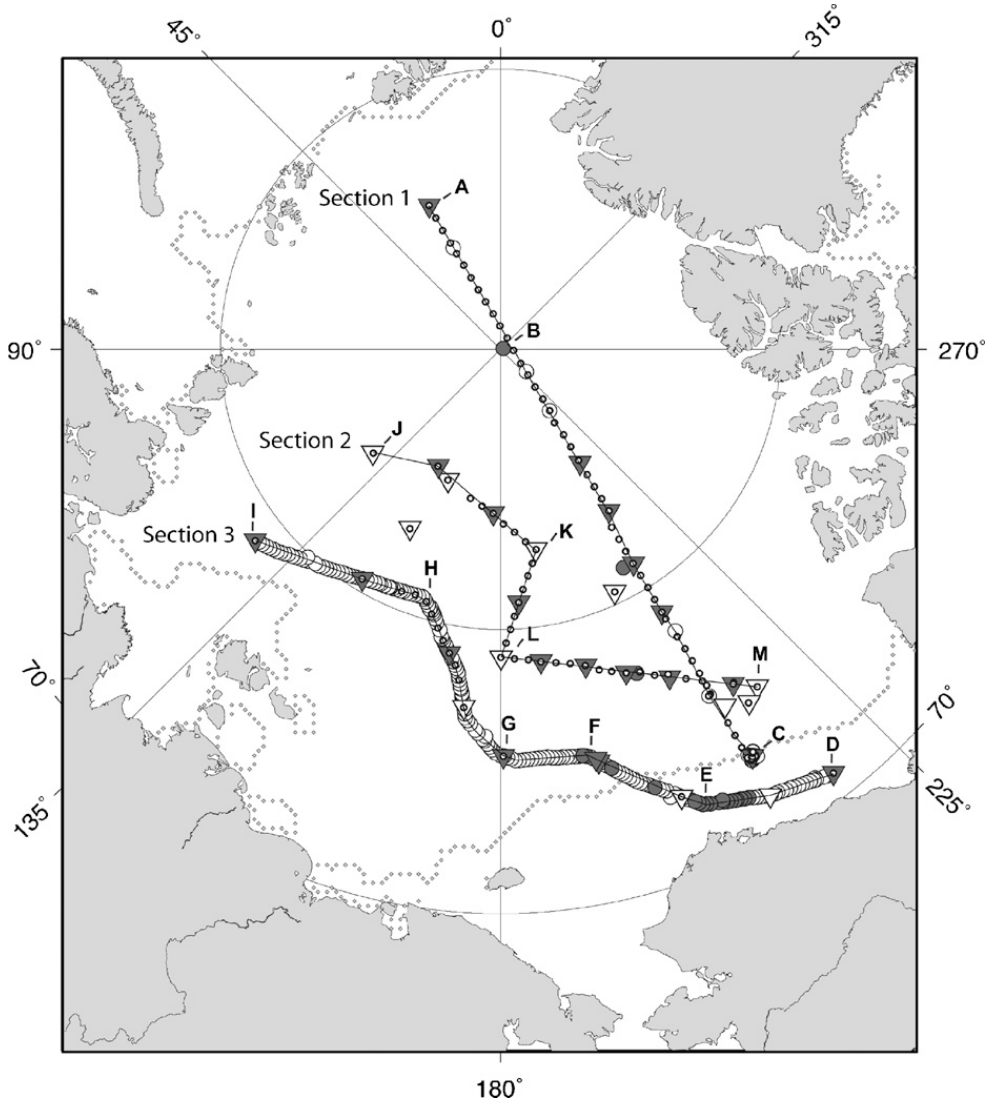

Fig. 1. Station map. Polar stereographic map of the Arctic Ocean and environs illustrating the sampling sites and the location of XCTD deployments. Land masses are in solid gray and the average sea ice extent for September 1997 is indicated by the dotted line of small gray diamonds. XCTD deployments are indicated by the smallest open circles. Sites at which samples were collected at only one or two depths are indicated by the larger circles. Sites of depth profiles (3–8 depths) are indicated by inverted triangles. Shaded symbols indicate stations at which samples were collected for chlorophyll *a*, prokaryotes, and viruses. Open symbols indicate stations at which samples were collected for chlorophyll *a*, but not for prokaryotes or viruses. Stations connected by solid lines make up three vertical sections illustrated further in Fig. 3. Way points are lettered in the order they were visited and indicate the beginnings and ends of the sections as well as points of significant deviations from a straight transect.

conductivity, temperature, and depth (UISSXCTD, hereafter referred to as XCTD; Sippican, Inc.). The third was analysis of discrete water samples collected by a through-hull sampling system. Discrete water samples were analyzed for salinity, dissolved oxygen, nutrients, chlorophyll *a* and other microbial variables as described below.

Samples were collected along transects at the cruising depth of the submarine and at discrete depths for vertical profiles at selected stations across the Arctic Ocean basin (Fig. 1). The through-hull sampling system consisted of Teflon® tubing reinforced with an outer stainless steel braided sheath that tapped into a seawater intake line as close as possible to the hull. Flow was controlled by a series of stainless steel needle valves. Sampling depth was controlled by changing the ship's position in the water column and ranged from 5 to 235 m. Depth profiles were collected by "spiral casts" (Muench et al., 2000) in which the ship spiraled downward through the water column to selected depths. Typical profiles consisted of 3–8 depths, which always included 55, 131, and 235 m. Four to five additional depths between 55 and 235 m were

sampled at selected stations to capture samples from hydrographic features identified from the XCTD data. All samples in a spiral cast were collected within a 0.4–3 km (mean 1.3 km) horizontal distance of the geographical position at which the sampling began.

To minimize potential cell damage due to rapid pressure drop across a needle valve, samples for microscopy were collected in polypropylene bottles nested within a stainless steel pressure vessel installed in the sampling line. Water entering the vessel flowed via silicone tubing into the bottom of the sample bottle. When the bottle was full, excess water spilled over the lip into the bottom of the pressure vessel and was forced by internal pressure to exit via a stainless steel outlet tube. During flushing and sampling, valves upstream of the pressure vessel were opened fully and flow was regulated with a valve downstream of the vessel to approximately 1 dm^{-3} min^{-1}. Three bottle volumes of seawater were flushed through the sampling vessel prior to shutting the inlet. The outlet was then kept slightly open to allow a slow bleed of pressure. The sample collection bottle was removed from the pressure vessel, capped, and kept at −1 °C until fixing with formaldehyde (typically within 1 h, always less than 4 h). One-hundred cm^3 aliquots were preserved with 0.2 μm-filtered, unbuffered formaldehyde (2% final w/v) and stored at 4 °C in 125 cm^3 capacity fluorinated polyethylene bottles.

2.2. Epifluorescence counts

Prokaryotes (members of the domains *Bacteria* and *Archaea* were not distinguished) and viruses were enumerated aboard the ship by epifluorescence microscopy using the SYBR Green I method (Noble and Fuhrman, 1998) with modifications as follows: SYBR Green I was prepared fresh daily by diluting the stock 1:1000 in phosphate-buffered saline, pH 7.4 (PBS). Subsamples of seawater (5–10 cm^3) were filtered onto 0.02 μm Anodisc filters (Whatman) using glass frit supports and no backing filter. When just dry, filters were removed from the frits with the vacuum still on, then placed face up on a 60 mm^3 drop of diluted SYBR Green I in a polystyrene petri dish and incubated in the dark at ambient temperature for 30 min to an hour. After staining, excess stain was removed by blotting the back of the filter on a lint free wiper (KimWipe®, KimberlyClark). Filters were placed on glass slides and covered with 35 mm^3 of mounting buffer (50% glycerol and 0.1% ascorbic acid in PBS) on a cover slip. Acridine orange staining (Hobbie et al., 1977) was also used to count prokaryotes in selected parallel samples for comparison with counts using the SYBR Green I method. Acridine orange was added to samples at a final concentration of 0.005%. Samples were incubated for 10 min, then filtered onto black, 0.2 μm pore-size, polycarbonate membranes (Poretics). Additional slides for SYBR Green counts were prepared from some of the preserved samples after storage at 4 °C for varying lengths of time in order to assess the effect of storage time on prokaryote and virus counts.

2.3. Contact rates

The specific contact rate of viruses with prokaryotes was calculated following Murray and Jackson (1992) as $2Sh\pi dD_vV$, where Sh is the Sherwood number, d the prokaryote diameter, D_v the diffusivity of the viruses in seawater at 0 °C (4.4×10^{-12} m^2 s^{-1}), and V the viral concentration. The value chosen for Sh (1.01) assumes nonmotile prokaryotes. The diameter of prokaryotes was assumed to be ca. 0.5 μm as derived from volume data reported by Sherr et al. (1997) for bacteria in the surface waters of the Arctic Ocean. The diffusivity of viruses at 0 °C was calculated as $D_v = kT/3\pi\mu d_v$, where k is the Boltzmann's constant, T the temperature in Kelvin (273 K), μ the viscosity of seawater at 0 °C (1.8 kg m^{-1} s^{-1}), and d_v the viral diameter (assumed here to be 50 nm).

2.4. Biomass estimates

The biomass of phytoplankton was estimated from measurements of chlorophyll using carbon:-chlorophyll ratio of 30 which is the average of the mean ratios of 28 and 31 calculated empirically for Arctic Ocean assemblages by Booth and Horner (1997) and Sherr et al. (2003), respectively. Biomass of heterotrophic prokaryotes was estimated from epifluorescence counts assuming 20 fg C $cell^{-1}$ (Lee and Fuhrman, 1987) following the results of Sherr et al. (1997) for Arctic prokaryoplankton. Viral biomass was estimated by assuming that an average virus contains 55 attograms of DNA (Steward et al., 2000), that viruses are on average 50% DNA and 50% protein by mass (i.e. similar to the ratio for tailed bacteriophages of the Order *Caudovirales*), and that C:protein and C:DNA mass ratios are both approximately 0.5. These assumptions yield a rough

estimate of 0.055 fg C virus^{-1}. Integrated biomass was calculated by simple trapezoidal integration.

2.5. Electron microscopy

Prokaryotes from some of the formaldehyde-preserved samples were prepared for transmission electron microscopy by the direct centrifugation technique (Suttle, 1993) except that centrifugation speed and time were reduced to 25 000 rpm for 30 min in an SW41 rotor (Beckman–Coulter). Samples were stained with 0.5% uranyl acetate for 20 s, destained 3 × 10 s in purified water (Milli-Q®, Millipore) then air-dried. Grids were examined in an electron microscope at 100 kV accelerating voltage. Prokaryotes (500 or 750/sample) were scored for the presence of intracellular viruses and for the presence of apparent capsular metal precipitation. Viral mortality of prokaryotes (expressed as percentage of heterotrophic prokaryotic production) was estimated by the procedure of Proctor and Fuhrman (1990) using the improved model equations derived by Binder (1999) with the assumptions that viral latent period equals prokaryote generation time and that grazers do not discriminate between infected and uninfected cells.

2.6. Data analysis and presentation

Counts of prokaryotes using the SYBR Green I and acridine orange staining protocols were compared by correlation and model II linear regression. The significance of the effect of storage time on abundance estimates was tested by ANOVA followed by post-hoc tests of the significance of differences in abundance between storage times. Correlations among variables were determined after log transformation to compensate for nonnormality in the distribution of values. XCTD data were smoothed using a three-point running average. Maps and two-dimensional graphs were created using the Generic Mapping Tools software (Wessel and Smith, 1991). Electron micrographs were captured on film and the number of intracellular viruses estimated by examination of the negatives on a light table. For publication, selected negatives were digitized on a flat-bed scanner with a transparency illumination unit. The resulting grayscale digital images were enhanced with the software Photoshop (Adobe Systems Incorporated) by adjusting brightness and contrast and input–output curves as needed to enhance visibility of intracellular viruses. Length-based volume units (mm^3, cm^3, dm^3, and m^3) are used throughout for volumes and concentrations following the well reasoned exhortation of Williams (2004).

3. Results

3.1. Analysis of SYBR Green I counts

Prokaryote counts made by the SYBR Green I or acridine orange methods were strongly correlated ($r = 0.96$, $p < 0.0001$, $n = 35$) and a model II linear regression resulted in a slope of 0.95 ± 0.1 (slope ± 95% C.I.), which was not statistically distinguishable from unity (Fig. 2(A)). Counts of prokaryotes and viruses determined for samples stored at 4 °C for 3–5 d prior to slide preparation were not significantly different ($p > 0.05$) from those prepared on the day of collection (Fig. 2(B)). Slides prepared from samples stored for approximately 2 weeks or more were significantly lower ($p < 0.001$). Prokaryote concentrations were reduced to an average of 52 ± 9% (mean ± s.d.) of initial values in 27 d ($n = 7$), and 40 ± 9% after 215 d ($n = 6$). Virus concentrations declined to 56 ± 7% of initial values within 16–21 d and 17 ± 13% after storage times ranging from 193 to 221 d.

3.2. Abundance and biomass of prokaryotes, viruses and phytoplankton

Over the area sampled during SCICEX 97, prokaryotes ranged from 0.04 to 0.5×10^9 dm^{-3} ($n = 155$) and viruses from 0.7 to 10×10^9 dm^{-3} ($n = 151$), as determined by the SYBR Green I staining method. The virus:prokaryote ratio ranged from 5 to 70 with an average of 26 ± 10 (mean ± s.d., $n = 151$) and specific contact rates ranged from 1 to 13 viruses prokaryote^{-1} d^{-1}. Chl *a* concentrations ranged from below detection (<0.001) to 0.2 μg dm^{-3}. Concentrations of bacteria, viruses, and chl *a* generally declined with depth and were horizontally variable at all depths (Fig. 3). The concentrations for all three groups were highest in the vicinity of the ice edge in the Beaufort and Chukchi Seas and lowest at more northern stations farthest from the ice edge. Correlations between log-transformed concentrations of bacteria and viruses ($r = 0.66$, $n = 150$), bacteria and Chl *a* ($r = 0.64$, $n = 155$), and viruses and Chl *a* ($r = 0.470$, $n = 139$) were all statistically significant ($p < 0.001$).

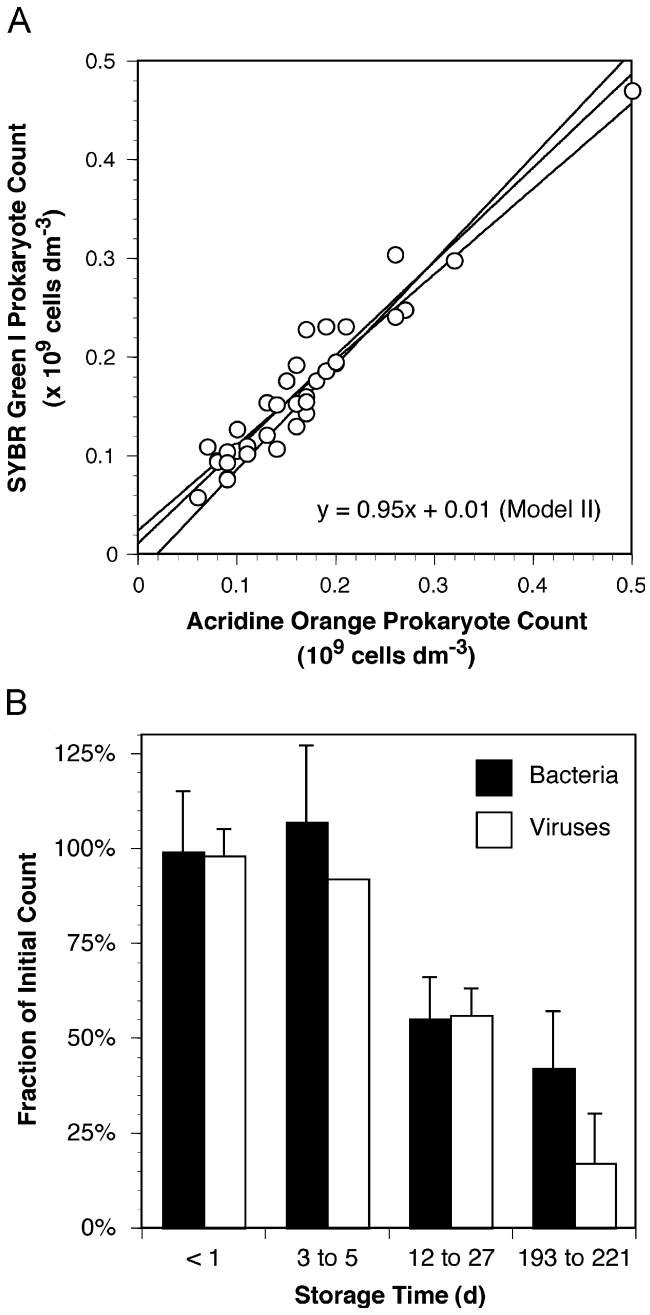

Fig. 2. Evaluation of prokaryote and virus counts. (A) Correlation of prokaryote counts obtained by the SYBR Green I and the acridine orange staining methods. The lines plotted are a model II regression line and the 95% confidence intervals for the slope, which is not significantly different from one. (B) Replicate counts of prokaryotes and viruses in formaldehyde-fixed samples after storage for varying intervals of time at 4 °C in the dark. Counts are expressed as a percent of the mean value at $T = 0$ d. Error bars indicate the standard deviation among different samples stored for the same interval of time.

Integrated (55–235 m) biomass estimates (Fig. 4) were determined at 31 stations for phytoplankton ($Phyto_{IB}$; 5–200 mgC m^{-2}) and at 20 stations for prokaryotes (Pro_{IB}; 270–850 mgC m^{-2}) and viruses (Vir_{IB}; 21–53 mgC m^{-2}). A plot of salinity at 55 m illustrates the relationship between biomass distributions and the origins of overlying water masses. Biomass for all three groups was highest along the shelf break, with the major peak occurring due north of the Bering Strait. Lowest values were observed in the central Arctic Ocean. A peak in Pro_{IB}, but not $Phyto_{IB}$ or Vir_{IB}, was also observed for one station at the northern edge of the Laptev Sea. Pro_{IB} and Vir_{IB} were each significantly correlated with $Phyto_{IB}$ ($r = 0.70$ and 0.67, respectively; $p < 0.001$), and Vir_{IB} was significantly correlated with Pro_{IB} ($r = 0.80$, $p < 0.0001$).

3.3. Viral infections of prokaryotes

Electron microscopy revealed viral infections in a variety of prokaryote morphotypes (Fig. 5). The number of intracellular viruses per infected cell ranged from 5 to >200 with an average of 35±33 (mean±s.d., $n = 45$). The number of viruses that could be discriminated within cells that appeared to be fully packed ranged from 8 to 200 with an average of 45±38 (mean±s.d., $n = 45$). The frequency of visibly infected prokaryotes ranged from below detection (<0.2%) up to 1.4% with an average for all samples of 0.5±0.4% (mean±s.d., $n = 26$). Converting to mortality estimates using the model of Binder (1999) suggests that on average 4% (range <1–11%) of the heterotrophic prokaryote production was being lysed by viruses at the time of the study.

3.4. Putative metal-precipitating prokaryotes

Prokaryote-sized cells surrounded by fibrous-looking rings or meshes of electron-dense material were observed by transmission electron microscopy (Fig. 6). These prokaryotes were found in 8 of 13 samples from a variety of stations (see Fig. 4(C)) and made up from 0% to 1% of the community with an overall average of 0.34±0.42% (mean±s.d., $n = 13$).

4. Discussion

4.1. Counts by epifluorescence microscopy

Our prokaryote abundance data are generally consistent with data from previous studies in this region (Sherr et al., 1997), but direct comparisons are not possible because of the different depth

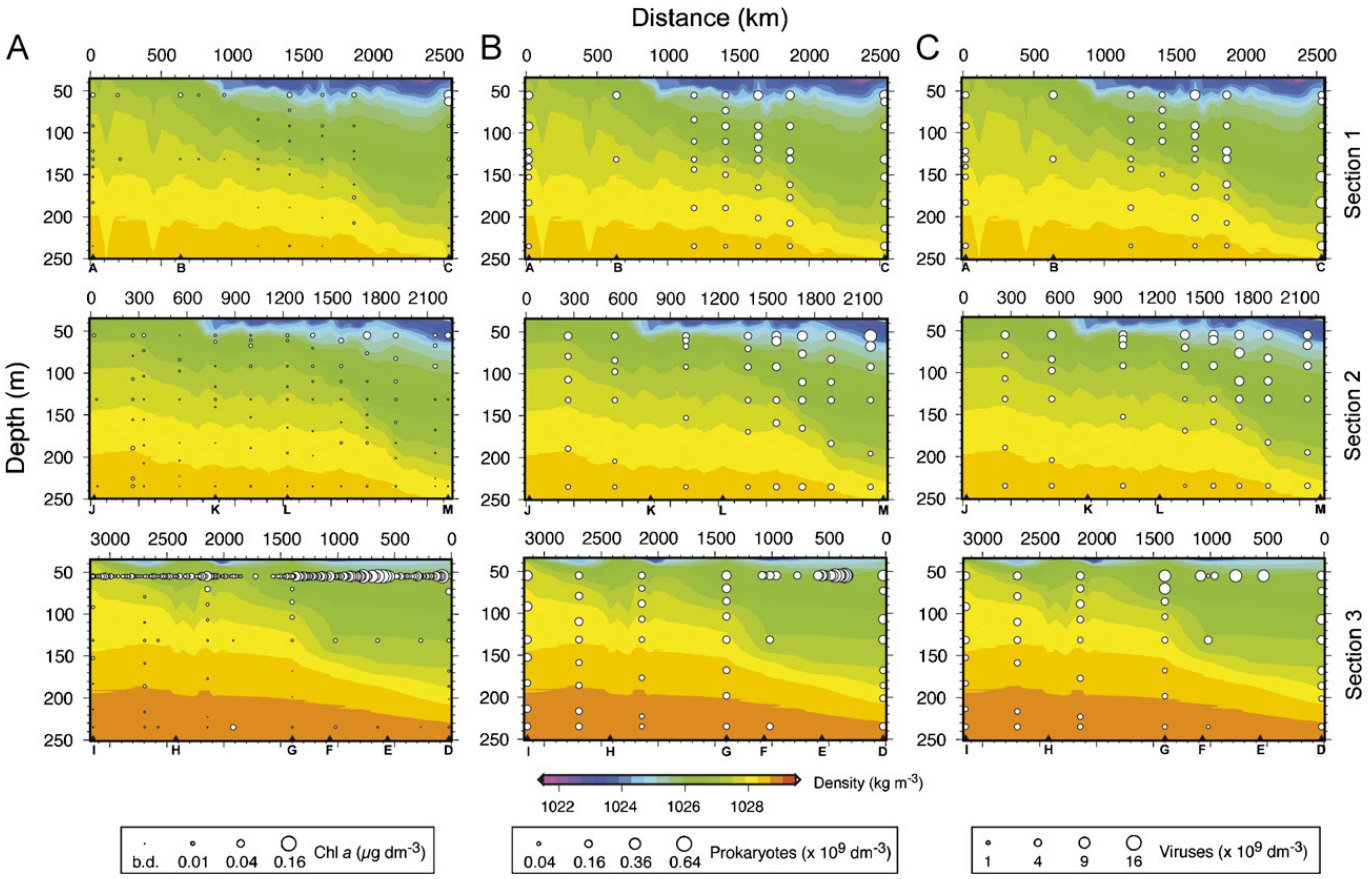

Fig. 3. Patterns of microbial abundance. Bubble plots in which circle area is proportional to (A) Chl *a* concentration, (B) abundance of prokaryotes, or (C) abundance of viruses are overlain on contour plots of seawater density plotted as distance vs. depth along three sections through the study area. Lettered markers at the bottom of each plot correspond to lettered way points in Fig. 1. Sections 1 and 2 correspond to Legs 1 and 2 of SCICEX 97 sampling nomenclature; Section 3 is a composite of Legs 3–5.

ranges studied. At the shallowest depth sampled in this study (55 m), higher values were associated with waters characteristic of the subsurface layer of the Arctic surface water over the Canada Basin compared to the halocline and the deeper Atlantic-derived water masses.

Although there are no previous data on viral abundance in the central Arctic Ocean, many of the concentrations reported here are higher than the highest reported values for nearby coastal surface waters (Maranger et al., 1994; Middelboe et al., 2002; Steward et al., 1996; Yager et al., 2001). This is most likely explained by prolonged sample storage in the latter studies, which can result in underestimates of bacteria (Turley and Hughes, 1994) and viruses (Wen et al., 2004).

Most of the samples for this study were filtered for microscopy on the day of collection, though one set of samples was stored for 5 d. Our storage test indicated that counts of prokaryotes and viruses did not decline substantially within this time frame. The test was not designed to detect short-term losses of viruses that have been observed to occur on the order of hours (Wen et al., 2004), but it has been suggested that virus counts in larger volume fixed samples (>50 ml) are relatively stable for at least a day, perhaps due to a reduction of surface effects (Patel et al., 2007). Because our samples were stored as relatively large volumes (ca. 100 ml) in fluorinated polyethylene bottles, we expect that storage losses were probably minimal. The very strong correlation observed between counts of prokaryotes captured on 0.02 μm filters and stained with SYBR Green I and those captured on 0.2 μm polycarbonate filters and stained with acridine orange confirms similar observations made in temperate waters (Fuhrman and Noble, 1998) and suggests that, even in very low productivity polar waters, virus-like particles counted by SYBR Green I can be reliably distinguished from particles that have been traditionally counted as prokaryotes.

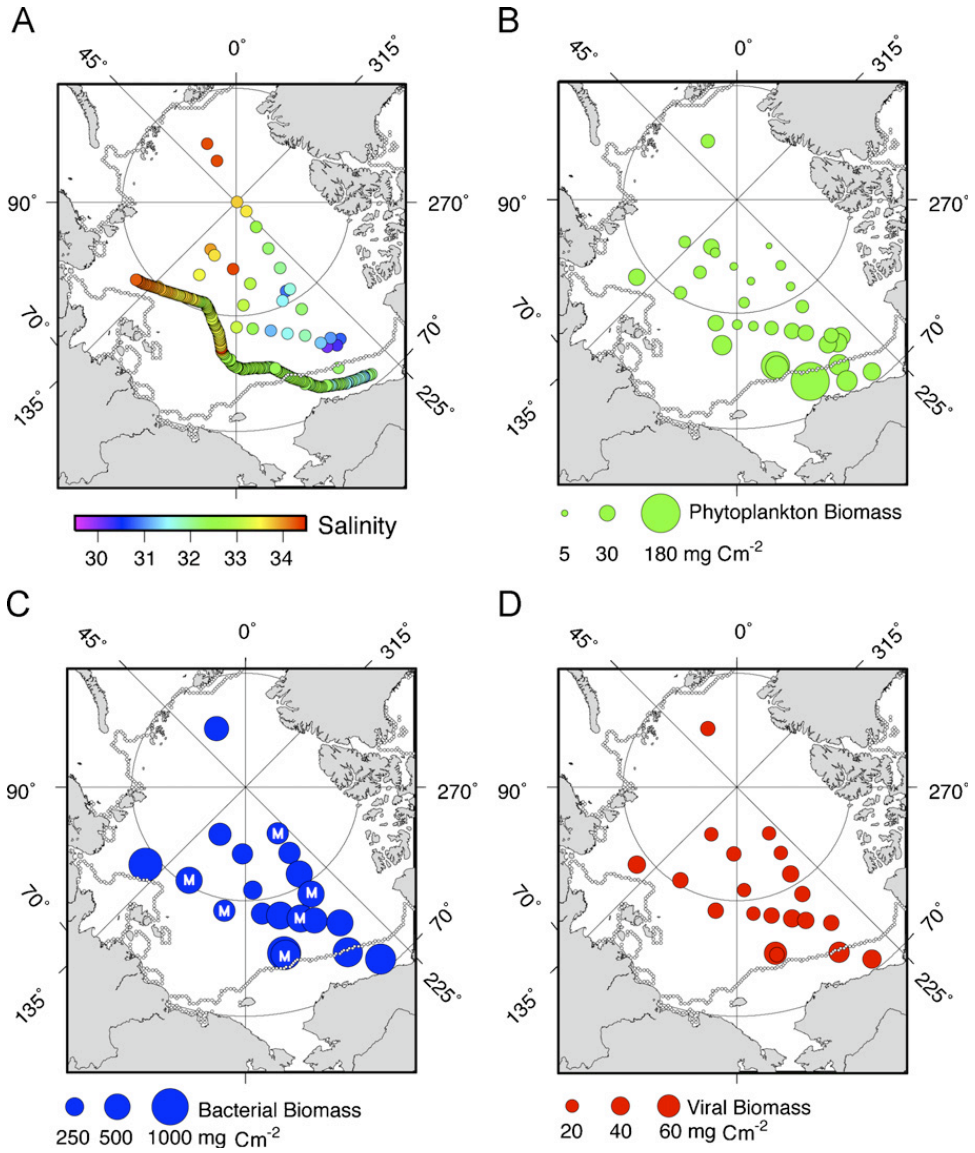

Fig. 4. Patterns of integrated microbial biomass. (A) Horizontal variability in salinity at 55 m is illustrated as color-coded circles and the integrated biomasses of (B) Chl *a*, (C) prokaryotes, and (D) viruses are illustrated as bubble plots where the areas of the circles are proportional to the biomass integrated from 55–235 m depth at each site. Symbols marked with an "M" in panel C indicate sites at which prokaryotes with putative metal-precipitating capsules were observed.

4.2. Integrated biomass

Because of sampling constraints of the submarine (minimum routine sampling depth of 55 m), we were not able to sample a region of potentially elevated microbial abundance near the underside of the ice. To get a more complete picture of the upper water column, we have combined the average of all of our integrated biomass estimates with the average of those for surface waters (0–50 m) from a previous investigation during summer over a similar range of latitude (Sherr et al., 1997). With these data we estimate average Pro_{IB}, $Phyto_{IB}$ and their ratio for the entire upper water column (0–235 m) of the Arctic Ocean Basin (Table 1). Data from two continental shelf stations reported on by Sherr et al. (1997) were excluded from this analysis. Averaging the oceanic data of Sherr et al. (1997) indicates that phytoplankton biomass exceeded that of prokaryotes in the upper 50 m with a Pro_{IB}:$Phyto_{IB}$ ratio of 0.6. For the 55–235 m range covered in this study, prokaryotic biomass was nearly the same as for the upper 50 m, but phytoplankton biomass was much less, resulting in an average Pro_{IB}:$Phyto_{IB}$ ratio of 10. Our estimate of viral biomass suggests that it was comparable to that of phytoplankton and ca. 6% of prokaryotic biomass when integrated over this deeper range. Over the entire combined

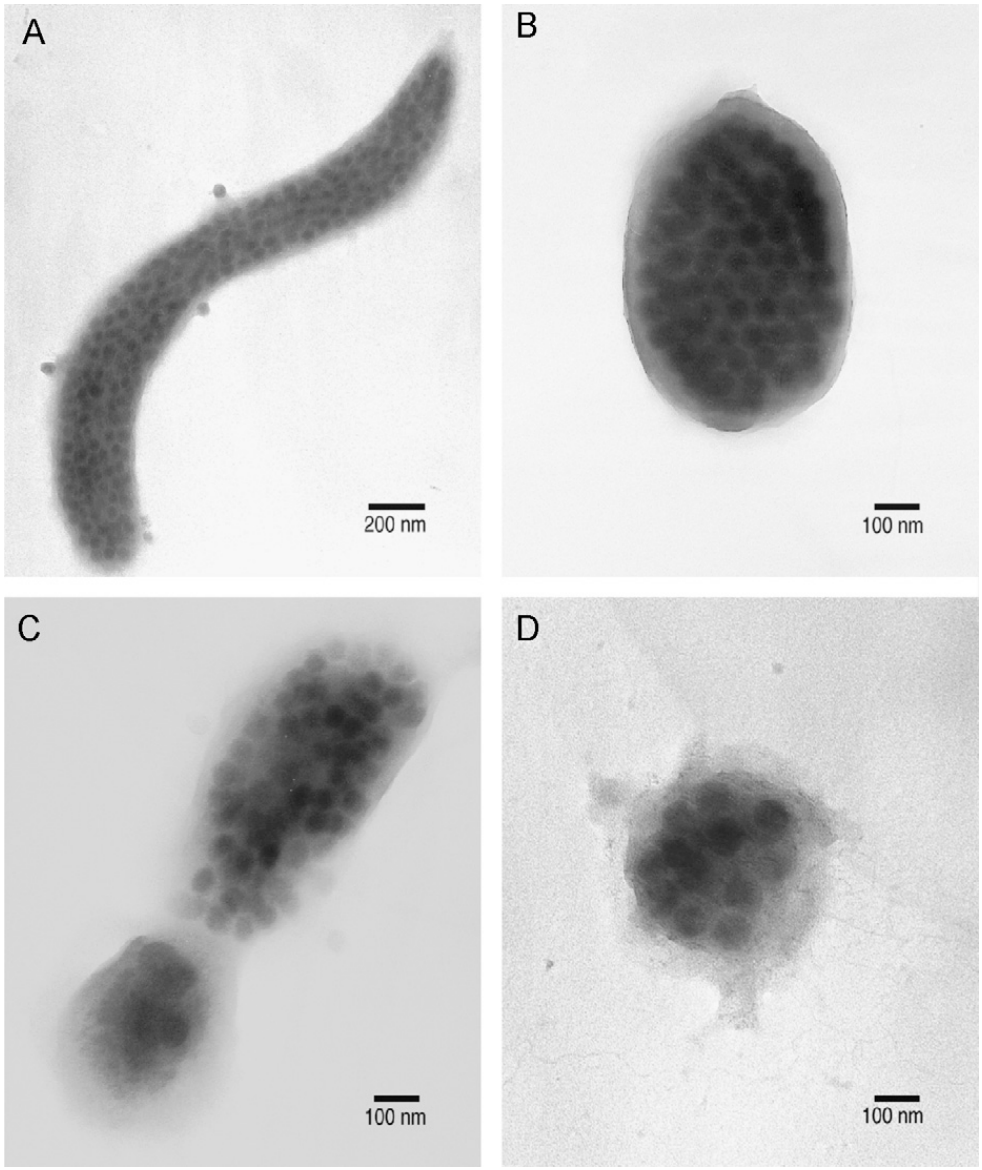

Fig. 5. Viral infections of bacteria. Transmission electron micrographs of infected prokaryotes illustrate a variety of cell morphologies and apparent burst sizes. (A) A long curving rod with several adsorbed viruses and >175 intracellular viruses, (B) an ovoid cell with about 55 distinguishable intracellular viruses, (C) an infected cell in the process of division with approximately 60 distinguishable intracellular viruses, and (D) an infected coccoid cell with about 13 intracellular viruses.

depth range (0–235 m), biomass estimates of prokaryotes and phytoplankton are roughly comparable. There is, of course, considerable uncertainty in these estimated basin-wide-average biomass ratios, since phytoplankton biomass was highly variable both horizontally and vertically, stations were not randomly distributed, and data are combined from different studies.

4.3. Viral infections of prokaryotes

Our estimate of the average infection rate of prokaryotes is the lowest yet reported for a marine or freshwater system. This is likely a result of the low virus concentrations and increased viscosity of cold arctic waters, which leads to specific virus–host contact rates that are an order of magnitude lower than calculated for typical temperate waters. That viral infections are able to persist under these conditions suggests that decreased contact rates may be compensated in part by reduced viral decay rates.

Though relatively few cells were infected, the mean number of viruses within cells that appeared to be fully packed, often taken as a very crude estimate of burst size, was nearly twice the average

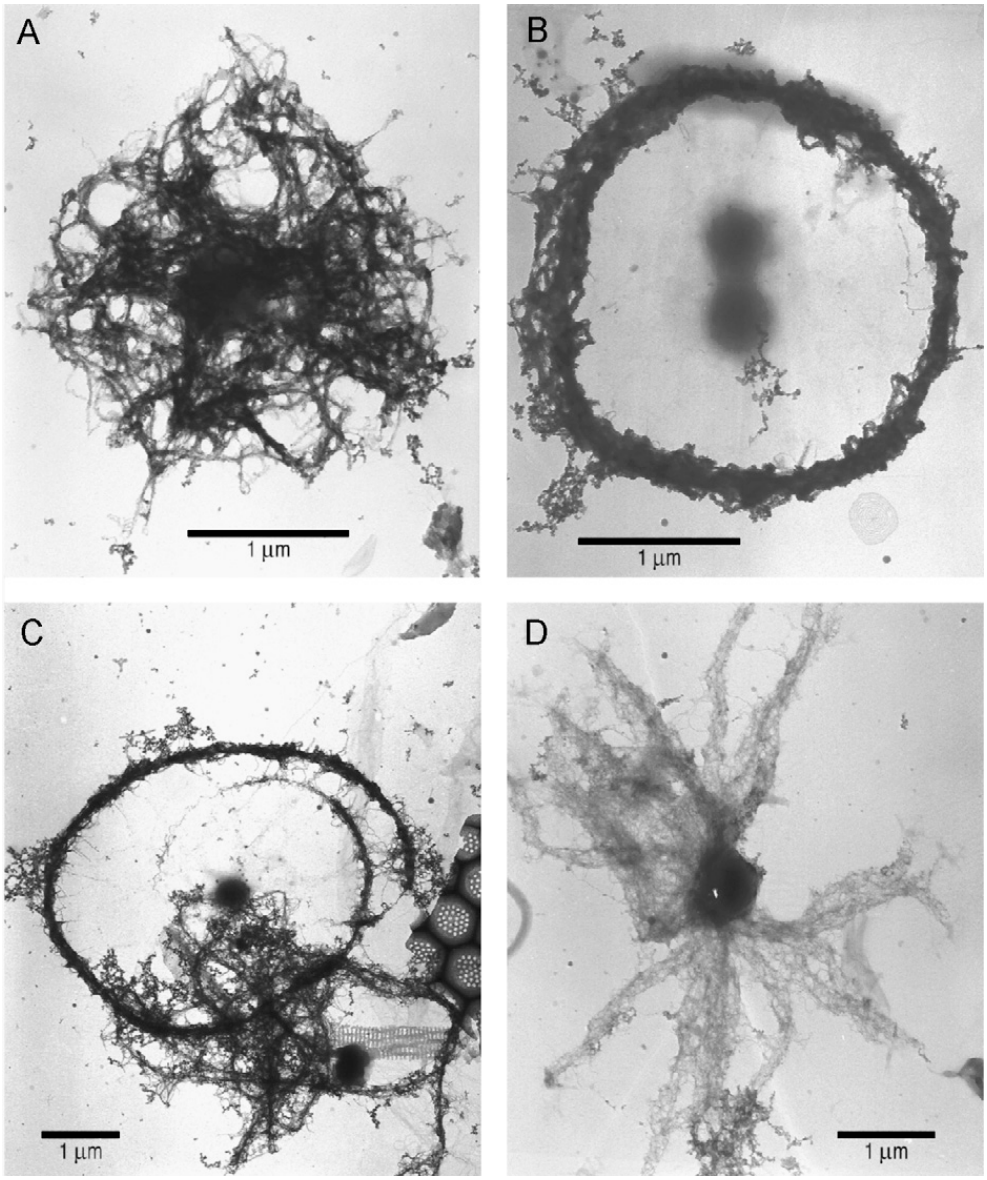

Fig. 6. Metal-precipitating bacteria. Transmission electron micrographs of prokaryotes with putative metal-precipitating capsules of differing morphology: (A) a cage-like capsule, (B) a thick ring-like capsule with a dividing cell, (C) cells with thin ring-like capsules, and a cage-like capsule next to a fragment of a diatom frustule, and (D) a capsule with radiating filaments. Sites at which cells of these types were found are indicated in Fig. 4(C).

Table 1
Average integrated biomass estimates of phytoplankton, prokaryotes, and viruses and the biomass ratio of prokaryotes and phytoplankton (Prok:Phyto) for all depth profiles in the central Arctic Ocean

Depth interval (m)	Average integrated biomass (s.d.) ($g\,m^{-2}$)			Prok:Phyto biomass ratio
	Phytoplankton	Prokaryotes	Viruses	
0–50	0.8 (1)	0.5 (0.1)	n.d.	0.6 (0.9)
55–235	0.04 (0.04)	0.5 (0.2)	0.03 (0.009)	10 (20)
0–235	0.8 (1)	1 (0.2)	n.d.	1 (2)

Values from 55–235 m are from this study and those from 0–50 m are calculated from data presented in Table 2 of Sherr and Sherr (1997) after excluding two shelf stations (Sta. 2 and 3). All values are rounded to one significant figure.

value of 24 calculated for marine bacteria in a recent review (Parada et al., 2006). The large average burst size and low frequency of infection observed in the Arctic is inconsistent with a reported positive cross-system correlation between burst size and frequency of infection (Parada et al., 2006), perhaps because the factors controlling burst size differ under more extreme conditions. Exceptionally low cell concentrations in the central Arctic Ocean, for example, could result in a low frequency of infection, but, according to one model (Abedon, 1989), should also select for larger burst sizes.

Our estimates of prokaryote mortality due to viruses in the central Arctic Ocean are consistent with a previous report on viral infections in the Chukchi Sea (Steward et al., 1996). Data from that study, reinterpreted using the equations of Binder (1999), indicated that viral mortality of prokaryotes decreased from an average of 15% in the highly productive waters of the southern Chukchi Sea to an average of only 6% in the Northern Chukchi Sea and was correlated with bacterial productivity. Our present data indicate that the viral contribution to mortality drops even further (to 4% on average) in the northern reaches of the central Arctic Ocean basin.

One troubling issue is the inability to account for much of the mortality of prokaryotes with estimates of grazing and viral lysis in these Arctic waters. For example, Steward et al. (1996), report that grazing and lysis accounted for $<25\%$, on average, of the total prokaryotic mortality in the Chukchi Sea. For the Arctic Ocean, Sherr et al. (1997) estimated that losses of prokaryotes due to grazing in surface waters ranged from 1% to 32% (mean 15%). The authors do not report on infection rates, but extrapolation from data in this paper and published infection data for Arctic Shelf waters by Steward et al. (1996) suggests that viral mortality of prokaryotes in the upper 50 m of the central Arctic is also likely to be low ($\leqslant 10\%$). If true, then estimates of mortality due to grazing plus viral lysis for the central Arctic Ocean, like the Chukchi Sea, would also be $\leqslant 25\%$, leaving the majority of mortality unaccounted for.

The imbalances appear to be too large to be explained as temporary deviations from steady state and suggest that the contributions of protistan grazing or viral lysis to mortality are underestimated or that other possible sources of mortality, such as autolysis or predatory bacteria, are being missed. This problem is not unique to polar waters, having been reported in coastal waters of Korea (Choi et al., 2003; Hwang and Cho, 2002). These apparent mismatches between production and mortality are in contrast to other reports that have found a reasonable balance between production and losses due to grazing and lysis combined (Fuhrman and Noble, 1995; Guixa-Boixereu et al., 1999) or found that losses that greatly exceeded production (Bratbak et al., 1992).

Using a dilution technique, viral lysis was found to account for 60–100% of mortality in Arctic coastal waters enriched in settling particles (Wells and Deming, 2006). Those data are few and from a somewhat different environment, but do suggest the possibility that the FVIC approach as applied in this study underestimated the contribution of viruses to the mortality of prokaryotes. If particle-associated prokaryotes were contributing disproportionately to secondary production or had higher rates of infection, then undersampling of those cells in the TEM surveys would lead to underestimates of the impact of viruses. It is also possible that not all of the assumptions used to convert FVIC to mortality apply in the cold oligotrophic environments. Further investigations are needed to better validate the mortality model assumptions and to constrain the mortality conversion factors for polar habitats.

4.4. *Metal-precipitating prokaryotes*

Surveys of metal-precipitating prokaryotes suggest that they are widespread in sediments (Cowen and Bruland, 1985; Nealson, 1983), in microbial mats around hydrothermal vents (Jannasch and Wirsen, 1981), in vent plumes (Cowen et al., 1986) and in the water column associated with particles (Cowen and Silver, 1984). Metal precipitates on the capsules of apparently free-living planktonic prokaryotes have also been observed as far north as the coastal waters of Norway (Heldal et al., 1996) and in the Chukchi Sea (G.F. Steward, unpublished observations). The present report extends the known range of these metal-precipitating planktonic prokaryotes to the central Arctic Ocean basin and suggests that this is a ubiquitous phenotype in the sea. The identities and diversity of genotypes presenting this phenotype in the marine pelagic zone is not known, but their possible biogeochemical significance can be inferred from the relevant literature.

The morphology of the ringed cells we observed in the central Arctic Ocean was strikingly similar to the Group III and IV Fe- and Mn-sequestering prokaryotes reported in Scandinavian coastal waters (Heldal et al., 1996). If we assume the composition of these cells is also similar and apply the per-cell Fe content of the rings from that report, we estimate that the equivalent bulk iron concentration associated with these prokaryotes ranged from <0.03 to 0.4 nM (average 0.1 nM). Concentrations of reactive iron in the central Arctic Ocean have ranged from 0.5 to 20 nM, but are typically 1–2 nM (Measures, 1999; Moore, 1983). The higher values were observed only in the shallowest waters and in the vicinity of sediment-laden ice (Measures, 1999).

Comparing our estimates of prokaryote-associated iron with the reactive iron concentrations from the previous studies suggests that these prokaryotes could account for up to 80% of the total reactive iron, though the contribution is probably less on average, perhaps $<10\%$. This range is similar to that reported for North Pacific waters for particle-associated metal-precipitating prokaryotes (Cowen and Silver, 1984). Regardless of its exact contribution to the total reactive iron pool, this precipitated iron is likely to cycle very differently from truly dissolved iron. The association of metals with prokaryotes on particulate material may be an important mechanism of selective scavenging and export to the deep sea and sediments (Cowen and Bruland, 1985). Conversely, iron precipitated on prokaryotic capsules represents localized high concentrations that may be readily ingested by heterotrophic or mixotrophic protists. Solubilization of the iron in acidic food vacuoles of protozoa and its subsequent assimilation or excretion could thus be an important source of soluble iron supporting primary productivity (Barbeau et al., 1996).

4.5. Summary and conclusion

We have presented an extensive survey of the concentrations of chlorophyll, prokaryotes, viruses, and of the incidence of viral infections of prokaryotes between 55 and 235 m across the Arctic Ocean. Prokaryotes made up a significant fraction of biomass in the upper 235 m of the Arctic Ocean, but because of low estimates of both viral and grazing mortality, the fate of this carbon remains uncertain. Observations of putative metal-precipitating prokaryotes suggest that grazing could be a potentially important pathway for the cycling of iron and manganese, but the consequences for productivity are unknown. Since the Arctic Ocean receives a disproportionately large fraction of terrigenous organic carbon (Benner et al., 2005), and the entire Arctic region plays a central role in scenarios of global change, an improved, quantitative understanding of these microbial processes and their influence on productivity and carbon remineralization in this remote region would be highly desirable.

Acknowledgments

We gratefully acknowledge J. Gunn and E. Quiroz for salinity and oxygen analyses. Essential logistical support was provided by J. Gossett, B. Campbell, and M. Mosher of the Arctic Submarine Laboratory and by the captain and crew of the USS *Archerfish*. We are indebted to W. Meier and the National Snow and Ice Data Center in Boulder, Colorado, for providing ice-edge data. This project was supported by National Science Foundation awards OPP 96-23130, OPP 96-25131, and OPP 96-17045.

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