

Population dynamics of bacterioplanktonic component associated with the phytoplankton biomass in Kongsfjorden, an Arctic fjord

Rupesh Kumar Sinha^{1,*}, K. P. Krishnan¹ and Savita Kerkar²

¹National Centre for Antarctic and Ocean Research, Head Land Sada, Vasco-da-Gama 403 804, India

²Department of Biotechnology, Goa University, Taliegao Plateau 403 206, India

Temporal variation (June and October 2012) in bacterial and phytoplankton communities of Kongsfjorden was studied using 16S rRNA gene clone libraries and marker pigments respectively. Proteobacteria was the dominant phyla in Kongsfjorden with Gammaproteobacteria (42%) and Alphaproteobacteria (84%) dominating in June and October, respectively. Retrieval of sequences affiliated to Verrucomicrobia, Gammaproteobacteria and Bacteroidetes in June corresponded with high autotrophic biomass (Chl *a*, 33 ng l⁻¹) whereas abundance of SAR 11 coincided with decrease in the intensity of autotrophic biomass (Chl *a*, 24 ng l⁻¹) in October. Thus, the distribution of bacterioplankton community varied with change in phytoplankton composition indicating a significant coupling between these two groups in the fjord water.

Keywords: Arctic, bacterial diversity, Kongsfjorden, phytoplankton pigments.

KONGSFJORDEN, located on the west coast of the Spitsbergen (Svalbard) archipelago at 79°N, is a glacial fjord in the Arctic¹. Due to its location as a border area between the Atlantic and Arctic biogeographical zones, Kongsfjorden has received much research attention in the recent past. The current interest of the researchers working on the fjord is primarily based on the fact that Kongsfjorden is highly suitable as a site for exploring the impacts of possible climate change, with both Atlantic water influx and melting of tidal glaciers being linked to climate variability¹. Information on bacterial assemblage at a given point of time could convey vital information pertaining to ecological aspects of the environment. Several factors have an impact on composition of the marine bacterial communities. Phytoplankton composition and thereby substrate availability and concentration seem to play important roles in shaping bacterial communities². Information on bacterial communities structure at the high biomass (autotrophic) zone of the fjord could provide insights on the taxonomy of members significant in bacterial–phytoplankton interactions. In this study,

variation in distribution of bacterial assemblages at depth of chlorophyll maxima is studied and discussed in relation to changes in composition of phytoplankton groups on a temporal scale.

Hydrographic observations and water sampling were conducted during June and October in 2012 at station K1 (78°54.299'N–12°13.665'E) near the Kongsbreen glacier front (Figure 1) using the research boat R.V. Teisten. A conductivity–temperature–depth (CTD) profiler (SBE 19 plus V2, Sea Bird Electronics, USA) equipped with a fluorescence sensor (Wet Labs, Philomath, USA) was used to obtain information pertaining to the hydrographic features (Figure 2) and fluorescence profile. Water samples were collected from a depth of 30 m showing primary chlorophyll maxima.

For the analysis of phytoplankton pigments, samples were immediately filtered on a GF/F filter (pore size 0.7 µm, Whatman, England) and analysed following the procedure of Van Hukelem³. Chl *a* concentrations were higher in June (33 ng l⁻¹) compared to those in October (24 ng l⁻¹). Peridinin (40 ng l⁻¹) was detected only in June, indicating the dominance of dinoflagellates in June while chl *b* (37 ng l⁻¹) was detected only in October. Fucoxanthin concentration was almost similar in June (20 ng l⁻¹) and October (19 ng l⁻¹), indicating the prevalence of diatoms in both the months.

DNA extraction from the microbial community collected over 0.22 µm polycarbonate membrane filter (Merck Millipore, Germany), was made using ultra clean soil DNA isolation kit (MOBIO, CA, USA). The 16S rRNA genes were amplified as described by Sinha *et al.*⁴ and cloned according to manufacturer's protocol (TOPO TA cloning kit, Invitrogen, CA, USA). The 16S rRNA genes in the plasmid were screened using a pair of specific primers, i.e. T3 (forward) and T7 (reverse) and amplified product was sequenced using an automated DNA sequencing system (Applied Biosystem, CA, USA) with BigDye™ Terminator v3.1 cycle sequencing kit (Invitrogen, CA, USA). Sequences were aligned using the Clustal W alignment function in the BioEdit software package (<http://www.mbio.ncsu.edu/BioEdit/bioedit.html>) and analysed using MOTHUR 1.34.4 program⁵. The 16S rRNA gene sequences of the clones obtained in June and

*For correspondence. (e-mail: kineto.magnetic@gmail.com)

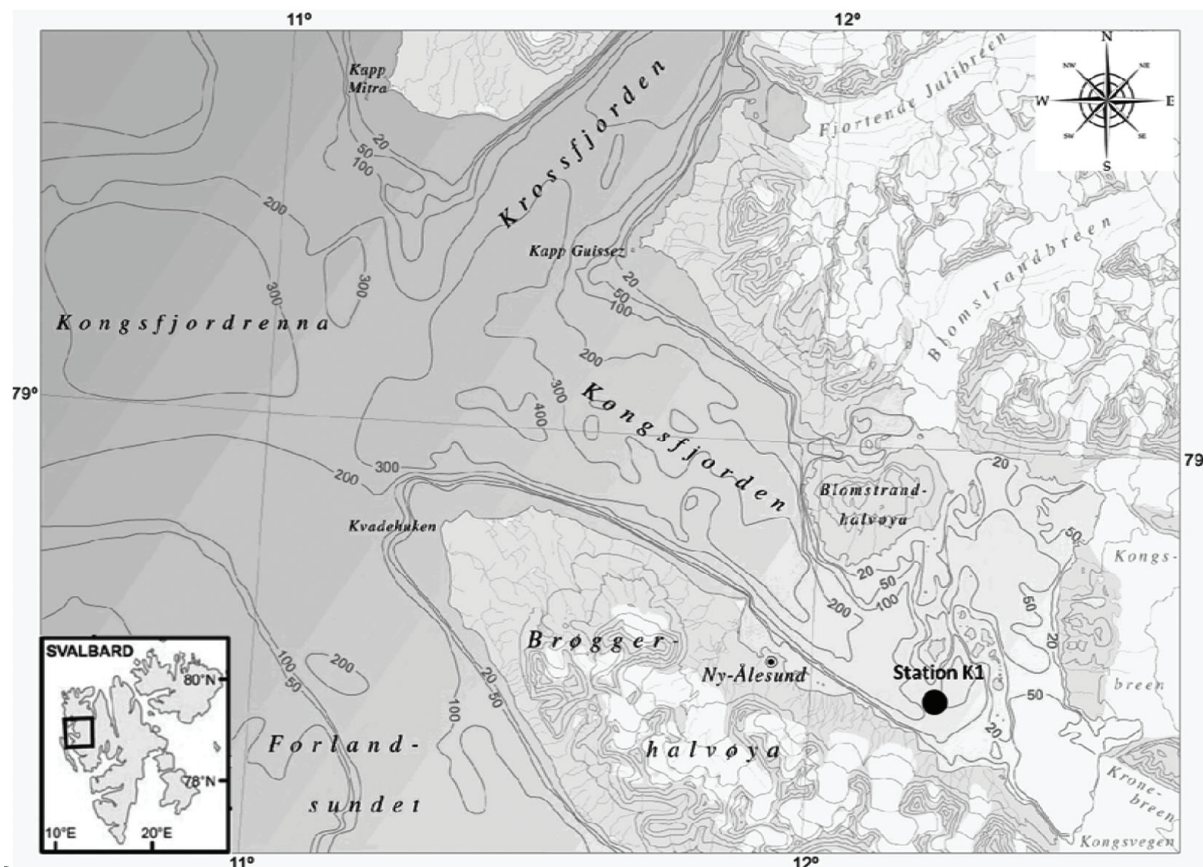


Figure 1. Location of sampling sites in Kongsfjorden, Ny-Ålesund in Svalbard, Arctic. The station K1 (●) represents the inner fjord (modified from Hop *et al.*¹).

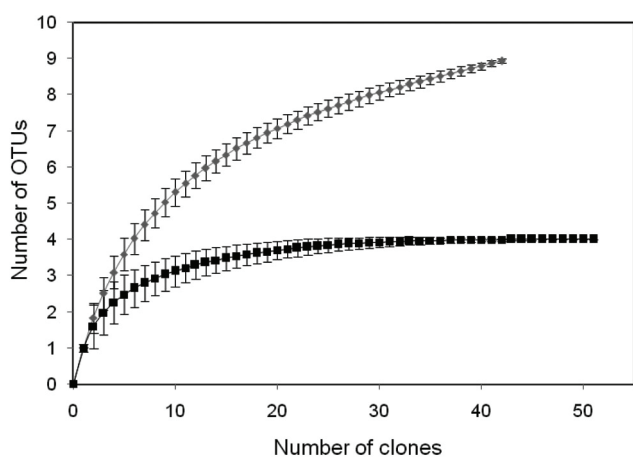


Figure 2. Rarefaction curves for June (—◆—) and October (—■—) clone library showing the number of clones sampled versus the number of operational taxonomic units (OTUs) within each library. Error bars represent the 95% CI.

October 2012 were deposited in EMBL (accession numbers LN852233–LN852327).

Based on the coverage estimates (Table 1 and Figure 2), about 95% and 99% of diversity was observed in the clone libraries for June and October samples respectively.

Clone library of June sample was dominantly represented by Gammaproteobacteria (42%) and Alphaproteobacteria (37%) while Bacteroidetes (19%) and Verrucomicrobia (2%) constituted the rest of the population (Figure 3 *a* and *b*). Most members of the Gammaproteobacteria were closely related to environmental clone sequences from glacier/fjord environments (Figure 4 *a* and *b*). Alphaproteobacteria was solely represented by SAR 11 clade of the genus *Candidatus Pelagibacter*. In October, Alphaproteobacteria (84%) was the dominant class followed by Gammaproteobacteria (10%) and Bacteroidetes (6%). The genus *Candidatus Pelagibacter* was the sole representative of alphaproteobacterial population in October also and most of the sequences were affiliated to those reported from Antarctic sea water (Figure 4 *a* and *b*).

Bano and Hollibaugh⁶ have found that SAR11 sequences accounted for 36% of all retrieved ribotypes in Arctic Ocean. Yager *et al.*⁷ reported SAR11 sequences in the DGGE profiles of samples taken before or after the peak of an algal bloom in the Chukchi sea while it was not detected during the peak of the bloom. Increase in abundance of SAR 11 with decrease in the intensity of autotrophic biomass in October (84%) as compared to that in June (37%) fortifies such observations. Stable carbon isotope probing experiments with sandy sediments in

Table 1. Biodiversity indices and statistics among the June and October clone libraries

Library	Individuals	OTUs	Chao1 (95% CI)	% Coverage	H' (95% CI)
June	43	9	10.0 (9.0–22.9)	95.3	1.9 (1.7–2.1)
October	52	4	4.0 (4.0–4.0)	99.8	1.0 (0.8–1.2)

Individuals, Number of clones sequenced; OTUs, Number of operational taxonomic units defined at 97% sequence identity; Chao1, Chao 1 nonparametric richness estimate with 95% confidence interval in parentheses; % coverage, Per cent library coverage based on Good's estimate, H' , Shannon–Weaver diversity index with 95% confidence interval in parentheses.

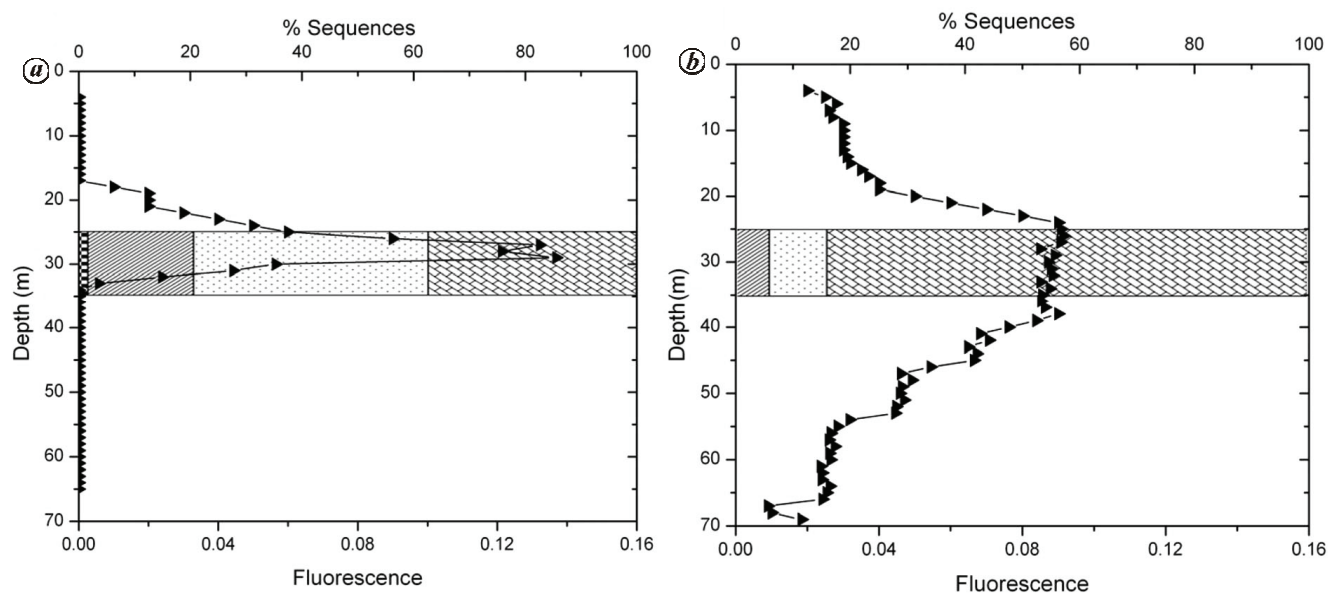


Figure 3. Percentages of 16S rRNA gene clone sequences (top x-axis) obtained in June (a) and October (b) at fluorescence (—▶—) peak (30 m) indicating high autotrophic biomass (bottom x-axis). Shown is the phylogenetic affiliation of class Alphaproteobacteria (▨), Gammaproteobacteria (▩), Flavobacteria (▤) and Verrucomicrobia (▧).

the Gulf of Mexico demonstrate that the gammaproteobacterial genera *Pseudoalteromonas* in addition to members of the Bacteroidetes assimilate algal extracts quickly⁸. The high abundance of Gammaproteobacteria (42%) in June with higher abundance of autotrophic biomass as compared to that in October (10%) is congruent with such observations.

Among the bacterial phyla that appear consistently in molecular surveys of Arctic marine environments, Verrucomicrobia are a widespread minority component in clone libraries from the marine water column⁶. Similarly we found that Verrucomicrobia represents a minor component (2%) of bacterial community in June. The free-living, epibiotic and symbiotic lifestyles of Verrucomicrobia with their predominantly carbohydrate-degrading metabolism indicate their potential role in mobilizing fixed carbon in a system⁹. A 16S rRNA gene-based bacterial community survey of the water column in an Arctic fjord of Svalbard yielded Verrucomicrobia as the dominant clone group which was associated with polysaccharide hydrolysis⁹.

Comparative 16S rRNA sequence analysis revealed that Flavobacteria is the dominant class of Bacteroidetes in marine picoplankton¹⁰. This is congruent with our observation where Flavobacteria constituted the complete Bacteroidetes population in June and October. High abundance has been linked to cold waters¹¹, phytoplankton blooms¹² and upwelling systems¹³. This distribution suggests a preference for more productive conditions. The higher autotrophic biomass observed in June along with higher incidence of Flavobacteria as compared to that in October (Figure 4 a and b) could be indicative of its preference for productive conditions.

To summarize, retrieval of sequences affiliated to Verrucomicrobia, Gammaproteobacteria and Bacteroidetes in June corresponded with the high autotrophic biomass (Chl *a*, 33 ng l⁻¹) whereas abundance of SAR 11 coincided with decrease in the intensity of autotrophic biomass (Chl *a*, 24 ng l⁻¹) in October. Thus, the observed change in the composition of bacterial community could be significantly linked to the variation in the phytoplankton assemblage in the fjord water. This study can serve as

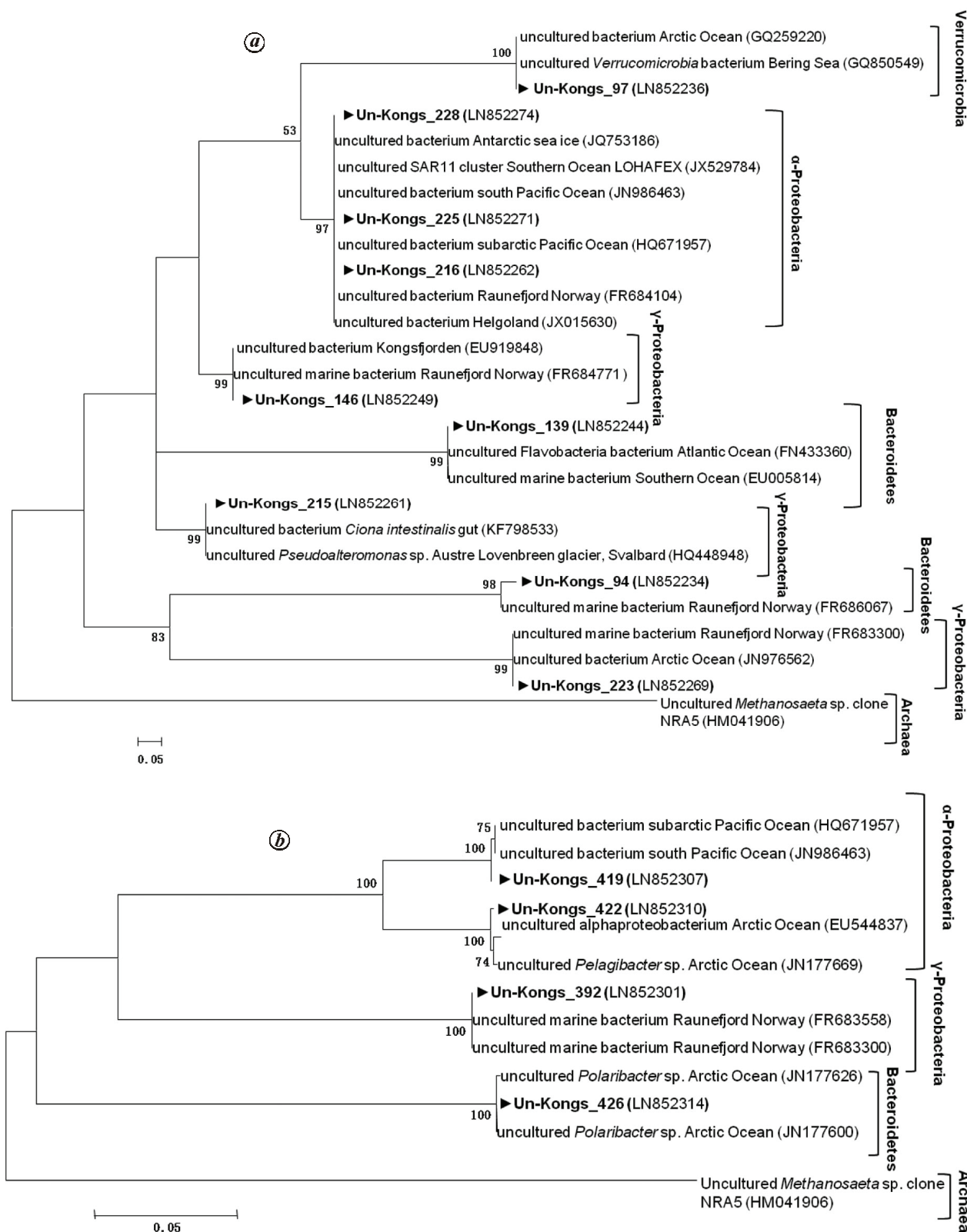


Figure 4. Phylogenetic tree based on 16S rRNA gene sequences showing the relationship of representative sequences for the clones obtained in June (a) and October (b) (prefixed with Un-Kongs) from the water samples at 30 m depth with their nearest phylogenetic neighbour sequences. Accession numbers of 16s rRNA gene sequences are denoted in parenthesis. Phylogenetic tree was constructed by maximum likelihood method. Numbers at nodes are bootstrap values. The bar represents 0.05 substitutions per alignment position.

basic information for future studies on spatio-temporal variation of bacterioplankton communities and their coupling with phytoplankton assemblage in Arctic fjords.

Conflict of interest: The authors declare that they have no conflicts of interest.

1. Hop, H. *et al.*, The marine ecosystem of Kongsfjorden, Svalbard. *Polar Res.*, 2002, **21**, 167–208.
2. Buchan, A., LeClerc, G. R., Gulvik, C. A. and González, J. M., Master recyclers: features and functions of bacteria associated with phytoplankton blooms. *Nat. Rev. Microbiol.*, 2014, **12**, 686–698.
3. Van Heukelem, L., HPLC phytoplankton pigments: sampling, laboratory methods, and quality assurance procedures. In *Ocean Optics Protocols for Satellite Ocean Color Sensor* (eds Mueller, J. and Fargion, G.), NASA Technical Memorandum, 2002, Revision 3, vol. 2, chap. 16, pp. 258–268.
4. Sinha, R. K., Krishnan, K. P., Hatha, A. A. M., Rahiman, M., David, D. T. and Kerkar, S., Diversity of retrievable heterotrophic bacteria in Kongsfjorden, an Arctic fjord. *Braz. J. Microbiol.*, 2017, **48**, 51–61.
5. Schloss, P. D. *et al.*, Introducing mothur: open-source, platform-independent, community-supported software for describing and comparing microbial communities. *Appl. Environ. Microbiol.*, 2009, **75**, 7537–7541.
6. Bano, N. and Hollibaugh, J. T., Phylogenetic composition of bacterioplankton assemblages from the Arctic Ocean. *Appl. Environ. Microbiol.*, 2002, **68**, 505–518.
7. Yager, P. L. *et al.*, Dynamic microbial response to an Arctic algal bloom at sub-zero temperatures. *Limnol. Oceanogr.*, 2001, **44**, 1882–1893.
8. Gihring, T. M., Humphrys, M., Mills, H. J., Huettel, M. and Kostka, J. E., Identification of phytodetritus-degrading microbial communities in sublittoral Gulf of Mexico sands. *Limnol. Oceanogr.*, 2009, **54**, 1073–1083.
9. Cardman, Z. *et al.*, *Verrucomicrobia* are candidates for polysaccharide-degrading bacterioplankton in an Arctic fjord of Svalbard. *Appl. Environ. Microbiol.*, 2014, **80**, 3749–3756.
10. Kirchman, D. L., Yu, L. Y. and Cottrell, M. T., Diversity and abundance of uncultured Cytophaga-like bacteria in the Delaware estuary. *Appl. Environ. Microbiol.*, 2003, **69**, 6587–6596.
11. Abell, G. C. J. and Bowman, J. P., Ecological and biogeographic relationships of class *Flavobacteria* in the Southern Ocean. *FEMS Microbiol. Ecol.*, 2005, **51**, 265–277.
12. Simon, M., Glockner, F. O. and Amann, R., Different community structure and temperature optima of heterotrophic picoplankton in various regions of the Southern Ocean. *Aquat. Microb. Ecol.*, 1999, **18**, 275–284.
13. Alonso-Saez, L. *et al.*, Bacterial assemblage structure and carbon metabolism along a productivity gradient in the NE Atlantic Ocean. *Aquat. Microb. Ecol.*, 2007, **46**, 43–53.

ACKNOWLEDGEMENTS. We thank the Director, ESSO-NCAOR. This work was undertaken as part of the project ‘Long term monitoring of Kongsfjorden system of Arctic region for climate change studies’. This is ESSO-NCAOR contribution number 48/2018.

doi: 10.18520/cs/v115/i9/1690-1694