

Heterotrophic bacterial and viral dynamics in Arctic freshwaters: results from a field study and nutrient-temperature manipulation experiments

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Abstract Heterotrophic bacterial and viral concentrations (range, 0.7×10^4 to $206.2 \times 10^4 \text{ ml}^{-1}$ and 0.05×10^6 to $128.9 \times 10^6 \text{ ml}^{-1}$, respectively) were determined in several Arctic freshwater environments, including lakes and glacial ecosystems (78.55°N, 11.56°E). Our bacteria and virus results mirrored trends seen in temperate lakes, with an average virus-to-bacteria ratio (VBR) of 13 (range, 7.3–25.2) and viral concentrations and DOC positively correlated with bacterial concentrations ($R = 0.964$, $P < 0.01$ and $R = 0.813$, $P < 0.05$, respectively). Lysogenic bacteria, determined by induction with Mitomycin C, were not detected in any of the investigated Arctic freshwater environments. Nutrient-addition experiments at in situ and at elevated temperatures were performed to elucidate the factors which influenced the bacterial growth and the virus–bacteria interactions in Arctic freshwaters. Our results suggest that multiple limiting factors interacted and constrained bacterial growth. Bacterial concentrations and doubling times increased at elevated temperatures and appeared to be co-stimulated by phosphorus and carbon.

However, viral concentrations showed a lack of response to nutrient addition thus indicating an uncoupling between bacteria and viruses in the experiment.

Keywords Bacteria · Viruses · Temperature · Nutrients · Arctic

Introduction

In Arctic freshwater ecosystems microbial life is pushed to its limits in regards to temperatures, light and nutrient levels (Ellis-Evans et al. 2001). These ecosystems have low trophic complexity and are mainly made up of the microbial loop where the bacterioplankton play a pivotal role as recyclers of the available nutrients (Laybourn-Parry 1997). Viral lysis of bacterioplankton disrupts this flow of energy and organic matter by forming a “viral loop” comprising bacteria, viruses and the dissolved organic matter pool (Thingstad 2000; Wilhelm and Suttle 1999; Wommack and Colwell 2000). Even though it is clear that viruses are a major cause of bacterial mortality in aquatic ecosystems (Wommack and Colwell 2000; Weinbauer 2004) there are only a few studies that report on viruses in polar freshwaters (Kepner et al. 1998; Laybourn-Parry et al. 2001; Lisle and Priscu 2004; Madan et al. 2005).

The interaction between a virus and its host is largely dependent on the physiological state of the host and the physico-chemical conditions of the aquatic environment (Lenski 1988; Miller 2001; Moebus 1996). There are two principal virus-host relationships; lytic or lysogenic. Lysogenised bacterial cells have the viral nucleic acid incorporated into the host genome as a prophage. The prophage may be induced into the lytic cycle, in response to environmental factors, and ultimately cause cell lysis and release

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new viral particles. Several studies have analysed the occurrence of lysogeny using the inductant Mitomycin C in both freshwater and marine bacterioplankton (Cochran et al. 1998; Jiang and Paul 1996; Lisle and Priscu 2004; Tapper and Hicks 1998; Weinbauer and Suttle 1996). The lysogenic cycle may provide a way for viruses to “survive” periods of low host abundance without driving their host to extinction (Wilson and Mann 1997; Wommack and Colwell 2000). One may therefore speculate that lysogeny may be a common phenomenon in polar freshwaters where the host is present in low abundance and is slowly growing.

The often observed positive correlation between bacterial and viral abundance in natural aquatic systems, indicates that these two components are closely linked and the parameters which control bacterial production may also influence viral processes (Weinbauer 2004; Wilhelm and Suttle 1999; Wommack and Colwell 2000). For this reason, one may predict that the factors influencing bacterial growth positively would also have a positive impact on viral processes. Phosphorus limitation of bacterial growth has been demonstrated in both freshwater and marine environments (e.g. Carlsson and Caron 2001; Elser et al. 1995; Farjalla et al. 2002; Granéli et al. 2004a). Also viruses may be particularly sensitive to phosphorus limitation, since viruses have a high nucleic acid to protein ratio, which could act as a potential control over lytic and lysogenic virus-host interactions (Wilson and Mann 1997).

Often experiments are performed to find a single limiting factor for bacterial growth, yet multiple limiting factors may interact and constrain bacterial growth (Pomeroy and Wiebe 2001). In the case of polar ecosystems one might anticipate that bacterial growth is limited by the interaction of low temperatures and low substrate concentrations (Wiebe et al. 1992). In the present study, we tested with independent experiments, if bacterioplankton growth in different Arctic freshwater environments was controlled by only one limiting factor, such as phosphorus concentration or if multiple limiting factors were involved, as has been suggested for the growth of marine heterotrophic bacteria (Pomeroy and Wiebe 2001). We also tried to elucidate if these multiple factors might influence virus and bacteria interactions in polar ecosystems. To accomplish this we conducted field studies in the Arctic and performed independent nutrient-addition experiments at in situ and also at elevated temperatures with natural viral and bacterial assemblages from the Arctic freshwater environment.

Materials and methods

Study sites and sample collection

Fieldwork was carried out near Ny-Ålesund, northwest Svalbard, (78.55°N, 11.56°E) (Fig. 1). Water from cryoconite holes, small meltholes that are formed on the glacier

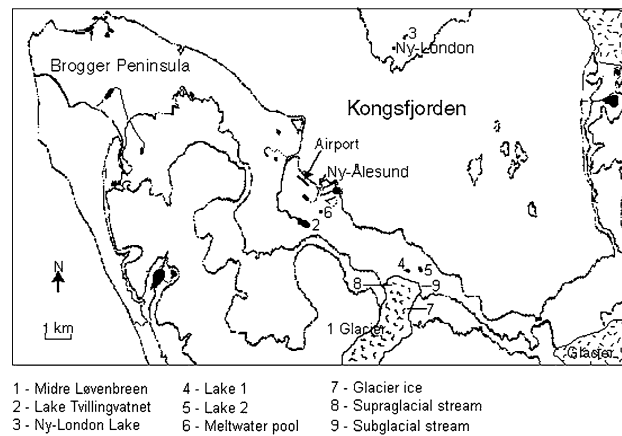


Fig. 1 Map of Kongsfjorden, Svalbard showing the location of the sampling sites. Map courtesy: J.C. Ellis-Evans

(Wharton et al. 1985), was collected from the Midre Løvenbreen glacier, Ny-Ålesund at around 200 m altitude during July and August 2002 and 2003. In 2003, samples were also collected from other freshwater environments around Ny-Ålesund which included: Lake Tvillingvatnet; a shallow lake near Ny-London (Ny-London Lake); two unnamed lakes situated close to the Midre Løvenbreen glacier (Lake 1 and Lake 2); a small pool of meltwater between Ny-Ålesund and Tvillingvatnet (meltwater pool); glacier ice collected close to the base of Midre Løvenbreen glacier and melted at ambient temperature (Glacier ice); a melt stream on Midre Løvenbreen (Supraglacial stream) and from water emerging from beneath the glacier (Subglacial stream). Duplicate 5-ml samples for viruses and bacteria enumeration were fixed in the field with 0.02 μ m pre-filtered glutaraldehyde (2% final concentration). Bacterial activity was measured in 2-ml microcentrifuge tubes incubated in situ at the surface of the lakes and cryoconite holes using the tritiated thymidine method (see below for details). Water samples and fixed samples were immediately transported, in the dark (<1 h), to the Natural Environment Research Council (NERC) research station, Ny-Ålesund where samples were processed, experiments set up and several parameters analysed as described in more detail below.

Analytical methods

Samples (~50 ml) for dissolved organic carbon (DOC) and dissolved nitrogen (DN) were filtered through GF/F filters (pre-ashed for 12 h at 550°C) or 0.2- μ m polycarbonate filters and immediately transferred into acid-washed bottles and stored at -20°C until analysed. DOC and DN were analysed by the Pt-catalysed (high-sensitivity catalyst) high-temperature combustion method using a Shimadzu TOCN-4100 total carbon and total nitrogen analyser. Chlorophyll *a* was extracted with 95% ethanol (Jespersen and Christoffersen 1987). Briefly, subsamples were filtered

through GF/C (47 mm) filters and stored at -20°C . Filtrates were then extracted in 5 ml of 95% ethanol at 4°C over night and then analysed on a Turner portable fluorimeter.

Enumeration of bacteria and viruses

Concentrations of bacteria from the 2002 season were measured with a FACSsort (Becton Dickinson) flow cytometer using the nucleic acid stain SYTO-13 (Molecular Probes, Invitrogen, Paisley, UK, del Giorgio et al. 1996). Samples from the season 2003 were analysed for viral and bacterial concentrations by filtering aliquots of samples to dryness onto 0.02 μm -pore-size Anodisc 25 membrane filters. Filters were stained with SYBR Gold nucleic acid stain (Molecular Probes, Invitrogen, Paisley, UK) and mounted on a microscope slide and a drop of *p*-phenylenediamine antifade placed on the surface and a coverslip added (Noble and Fuhrman 1998). Counts of stained bacteria and viruses were obtained at $1,000\times$ magnification, using a Zeiss Axioscop equipped for epifluorescence. Blue light excitation (filter set 487909 with 450–490 nm exciter filter, 510 nm chromatic beam splitter and 520 nm barrier filter) was used.

Bacterial production

Bacterial production was estimated by the microcentrifuge method as explained by Kirchman (2001). In brief, five replicate 1.7 ml samples were collected into 2 ml microcentrifuge tubes and [^3H]thymidine (84.0 Ci mmol^{-1}) (Amersham Biosciences UK, Little Chalfont, Bucks) was added to a final concentration of 30 nM. Duplicate control samples were immediately inactivated with 90 μl of 100% TCA. All the samples were then incubated for 90 min to allow the label to be incorporated into the bacterial biomass. After incubation, 90 μl of 100% TCA was added to all samples except for the duplicate inactivated controls. The tubes were then centrifuged at $16,000g$ at 4°C for 10 min, following this the supernatant was removed by aspiration. To each tube 1.7 ml ice-cold 5% TCA was added, the tube was then vortex mixed, centrifuged and the supernatant aspirated (as above). Finally 1.7 ml of ice-cold 80% ethanol was added, the centrifugation and aspiration steps repeated (as above) and 1 ml of scintillation cocktail (Ecoscint) added. Samples were then counted by liquid scintillation in a Beckman LS6500 scintillation counter. Thymidine incorporation was converted into bacterial carbon production by using a conversion factor of $2 \times 10^{18}\text{ cell mol}^{-1}$ (Bell 1993) and assuming a carbon content of 10 fg cell^{-1} (Bratbak and Dundas 1984).

Induction assay for lysogenic bacteria

The occurrence of lysogenic bacteria was estimated by monitoring the viral concentration in water samples treated

with a potent mutagen for prophage induction (the antibiotic Mitomycin C) and comparing it with untreated control samples (Paul and Jiang 2001). In short, triplicate water samples (15 ml) were either treated with $1\text{ }\mu\text{g ml}^{-1}$ Mitomycin C (Sigma, St Louis, MO, USA) or left untreated (controls) (Paul and Jiang 2001). The samples were incubated in the dark at room temperature (20°C) for 24 h and then fixed with 0.02 μm filtered glutaraldehyde (final concentration 2%) and analysed immediately for bacterial and viral concentrations using SYBR gold staining as detailed above. If there was a statistically significant increase in viral concentration in the Mitomycin C treatment relative to the control then this was an indication of presence of lysogenic bacteria.

Nutrient and temperature manipulation experiments

Cryoconite or lake water was filtered through 0.2- μm pore-size polycarbonate filters to remove grazers and bacteria. Additional water samples were filtered through a GF/F glass-fiber filter (0.7 μm nominal pore size) and the filtrate was used as a bacterial/viral inoculum. Batch cultures with enhanced nutrient concentrations (final volume of 200 ml) were created by adding a 5% bacterial/viral inoculum to 0.2 μm -filtered-water and nutrients added as described below. Each treatment was performed in triplicates in acid-washed (10% HCl and Milli-Q rinsed) glass bottles with a control consisting of 0.2 μm -filtered-water and a 5% bacterial/viral inoculum.

Nutrient solutions were made up with 0.2 μm -filtered Milli-Q water and added to the batch cultures. In 2002, batch cultures had a final concentration of 10 mg carbon (C) (glucose) per litre, 71 μM N (added as NaNO_3) and 3 μM P (added as KH_2PO_4) in the following combinations: either C, N, P or CNP. In 2003, batch cultures had a final concentration of 3 mg C (glucose) per litre, 3 mg Hu (humic substance), 10 μMN (added as NH_4NO_3) and 1 μM P (added as KH_2PO_4) in the following combinations: either C, Hu, N, P, CNP or HuNP. Humic substances were added in the form of fulvic acid extracted from Laurentian soils (Fredriks Research Products, Netherlands). A partial elemental composition of the fulvic acid, as given by the supplier, was C (43.2%), H (4%), N (0.27%), Fe (0.013%), and ash (<1.0%). The humic substance was added as an alternative, more refractory, carbon source relative to glucose. For comparison, in situ nutrient levels in Svalbard freshwater lakes may vary between 0.2 to 2 μM P and 17 to 119 μM N with a DOC concentration range of 0.2–1.9 mg l^{-1} per litre (Ellis-Evans et al. 2001). Significantly lower nutrient levels are associated with the water phase in cryoconite holes (0.02–0.05 μM $\text{PO}_4\text{-P}$, 0.02–1 μM $\text{NH}_4\text{-N}$ and 0.04–0.1 $\text{NO}_3\text{-N}$) (S awstr om et al. 2002). 2002 batch cultures from cryoconite water were incubated at 4°C in the dark for 216 h. Samples for bacterial

concentrations were collected 72, 120, 168 and 216 h after the beginning of the experiment and fixed with 0.02 μm -filtered glutaraldehyde (2% final concentration) and stored at 4°C in the dark and processed within 2 months of collection. In 2003 batch cultures from cryoconite and lake water were set up and incubated in the dark for 144 h. The cryoconite batch cultures were incubated at two different temperatures (0.2 and 12°C), whereas the lake water (Lake 1) was incubated only at 12°C. Lake water temperatures in Svalbard varies seasonally, with low winter temperatures of 2–3°C but high summer temperatures of up to 15°C (Ellis-Evans et al. 2001). The water temperatures in cryoconite holes are significantly lower with an average of 0.1°C (Sävström et al. 2007). Samples for bacterial and viral concentrations were fixed in 0.02 μm -filtered glutaraldehyde (2% final concentration), stored at 4°C in the dark and processed within 24 h.

Bacterial growth rates and doubling times

Bacterial doubling/generation times ($\ln 2/\mu$) in the 2002 cryoconite enrichment experiments were determined from the exponential growth rate (μ) (Kirchman 2001). The exponential growth rate (μ) was calculated from the slope of a least-squares regression of the natural log (\ln) bacterial concentrations as a function of time for each treatment (Guillard 1973). Initial bacterial concentration was not included when determining the exponential growth rate (μ). In 2003, bacterial doubling times were estimated by the following equation: doubling time = $(\log_{10}N_t - \log_{10}N_0)/\log_{10}2$, where N_0 = initial concentration in the diluted batch culture (20 \times dilution of the in situ concentration) and N_t = concentration at the end of the experiment (144 h).

Statistics

Statistical analyses were performed in SPSS (version 11.0.0 for Windows). For the Arctic field survey, Pearson correlations were examined between bacterial and viral concentrations, bacterial production and measured physical variables. Data from the nutrient and temperature manipulation experiment (2002 and 2003) were analysed using one-way ANOVA and the Tukey Post-Hoc test was used to determine the significance of differences among treatments. An independent samples *t* test was used to investigate differences between bacterial doubling times at the different incubation temperatures.

Results

Arctic field survey 2003

The low DOC and DN values ranged from 0.24 to 1.25 mg l^{-1} and 0.09 to 0.24 mg l^{-1} , respectively (Table 1).

Table 1 Chemical characteristics of different freshwater environments in Svalbard 2003

Environment	DOC (mg l^{-1})	DN (mg l^{-1})	Chlorophyll <i>a</i> ($\mu\text{g l}^{-1}$)
Lake 1	0.45	0.10	0.58
Lake 2	0.24	0.24	–
Lake Tvillingvatnet	0.28	0.11	1.06
Ny-London Lake	1.25	0.18	0.95
Cryoconite	0.42	0.09	0.08
Meltwater pool	1.19	0.17	1.07

All results are mean values of two replicates

Chlorophyll *a* concentrations were also low (0.08–1.07 $\mu\text{g l}^{-1}$) and all the freshwater environments were considered oligotrophic. Bacterial concentrations varied from 0.7 to 206.2×10^4 cells ml^{-1} and were positively correlated with DOC concentrations ($R = 0.813$, $P < 0.05$, $N = 6$). Variation in DOC, DN and chlorophyll *a* was 5, 2.5 and 13-fold, respectively, whereas variation in bacterial concentration was much higher (312-fold). Viral concentrations were always higher than bacterial concentrations (ranging from 0.05 to 28.9×10^6 cells ml^{-1}) and strongly positively correlated with bacterial concentrations ($R = 0.964$, $P < 0.01$, $N = 8$). Virus-to-bacterium ratios (VBR) varied from 7.3 to 25.2 with the highest VBR found in Lake 1. Bacterial production was low with the highest activity found in a melt pool near lake Tvillingvatnet (542 $\text{ng Cl}^{-1}\text{h}^{-1}$). Lysogeny was undetectable in the investigated environments (cryoconite hole, Lake 1 and Lake 2) (Table 2).

Nutrient and temperature manipulation experiments

2002 nutrient experiment

Bacterial concentrations in all the treatments ranged from 0.3 to 110×10^4 cells ml^{-1} . Highest bacterial concentration was found in the CNP treatment on the last sampling occasion (Fig. 2). Bacterial numbers were significantly higher in the CNP and P treatment relative to the control when all the sampling times were considered ($P < 0.05$, one-way ANOVA). However, there was no significant difference in bacterial concentrations between the CNP and P treatment. Bacterial exponential growth rates ranged between 0.2 (control) and 0.7 per day (CNP) and significantly higher rates were noted in all the amended treatments when compared to the control ($P < 0.01$, one-way ANOVA) (Fig. 2). The bacterial growth rates in the phosphate amended treatments (CNP and P) were over 3 times higher than the control.

Table 2 Mean values of bacterial and viral concentrations, VBR and bacterial production (BP) from different freshwater environments in Svalbard 2003

Environment	Bacteria ($\times 10^4 \text{ ml}^{-1}$)	Viruses ($\times 10^6 \text{ ml}^{-1}$)	VBR	BP ($\text{ng C l}^{-1} \text{ h}^{-1}$)	Viruses (control $\times 10^6 \text{ ml}^{-1}$)	Mitomycin C (% of the control)
Glacier ice	4.6 ± 0.7^b	0.3 ± 0.01^b	7.5	101 ± 61^d	–	–
Lake 1	59.1^a	14.9^a	25.2	92 ± 12^c	15.2 ± 0.4^b	90.0 NS
Lake 2	5.0	–	–	64 ± 58^d	0.52 ± 0.05^b	107.4 NS
Lake Tvillingvatnet	37.9	4.3	11.3	134 ± 24^d	–	–
Ny-London Lake206.2 ^a	28.9^a	14.0	226 ± 30^d	–	–	–
Cryoconite	1.9 ± 0.3^c	0.3 ± 0.02^c	13.6	106 ± 29^d	0.09 ± 0.01^b	102.9 NS
Meltwater pool	74.9^a	15.2^a	20.3	542 ± 22^d	–	–
Supraglacial stream	0.7^a	0.05^a	7.5	261 ± 68^d	–	–
Subglacial stream	11.6^a	0.9^a	7.3	–	–	–

Lysogeny induction results are shown in the last two columns, showing virus numbers in the untreated control (24 h incubation) and the percentage change in the Mitomycin C treatment compared to the untreated control. Standard errors are presented for $N > 2$. NS not significant

^a $N = 2$

^b $N = 3$

^c $N = 4$

^d $N = 5$

^e $N = 10$

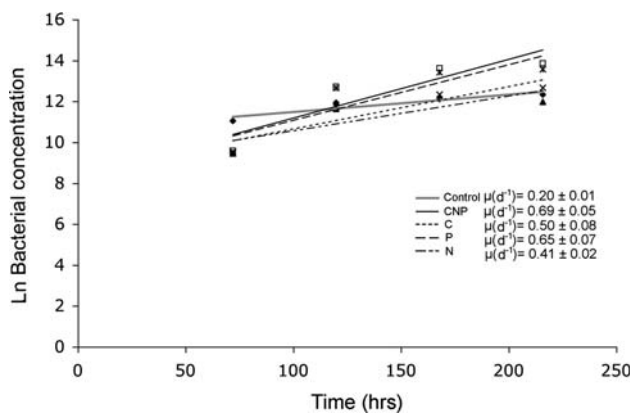


Fig. 2 Bacterial growth rate slopes from the 2002 cryoconite nutrient amended experiment. Exponential growth rates (μ) are mean of three replicates with standard errors. Treatments are no amendment (control filled diamond), glucose and nutrient addition (CNP open square), only glucose addition (C \times), phosphorus addition (P^{*}), or nitrogen addition (N filled triangle)

2003 nutrient and temperature experiment

A significant increase in viral concentration was noted in the cryoconite CNP treatment when compared to the control treatment ($P < 0.01$, one-way ANOVA). However, in general there was no significant change in virus concentrations after the addition of nutrients (Fig. 3a). Addition of nutrients (N, C and CNP at 0.2°C and P at 12°C) increased bacterial numbers in cryoconite water when compared to the control treatment (Fig. 3b). There was no significant difference in bacterial concentrations between the P and the

CNP treatment or the Hu and HuNP treatment in the cryoconite water (Fig. 3b). There was a significant reduction in bacterial numbers in the cryoconite water at 12°C with C addition when compared to the control (Fig. 3b). Bacterial concentrations were significantly higher in the lake water that had P, CNP, Hu and HuNP addition when compared to the control (Fig. 3b). There was a significant difference between P and CNP treatments in the lake water ($P < 0.01$, one-way ANOVA). VBRs were relatively similar in the treatments with cryoconite water (average of 12 ± 1 SE, Fig. 3c). However, in the lake water a significant decrease in VBR was observed in the P, CNP, Hu and HuNP treatment, which was largely due to the increase in bacterial numbers in these treatments. An increase in temperature, from 0.2 to 12°C , resulted in significantly higher bacterial concentrations in all the nutrient amended and control treatments of cryoconite water ($P < 0.01$, one-way ANOVA, Fig. 3b).

Bacterial doubling times in 2002 and 2003 experiments

Bacterial doubling times varied from 0.69 to 3.43 days (Fig. 4). In 2002, all cryoconite nutrient amended treatments at 4°C had significantly ($P < 0.01$, one-way ANOVA) shorter bacterial doubling times when compared to the control. The shortest doubling times were noted in the CNP and P treatment but there was no significant difference in the doubling times between the CNP and P treatment (Fig. 4). In 2003, significantly shorter doubling times were noted in the cryoconite C treatment at 0.2°C compared to the control ($P < 0.05$, one-way ANOVA). In the

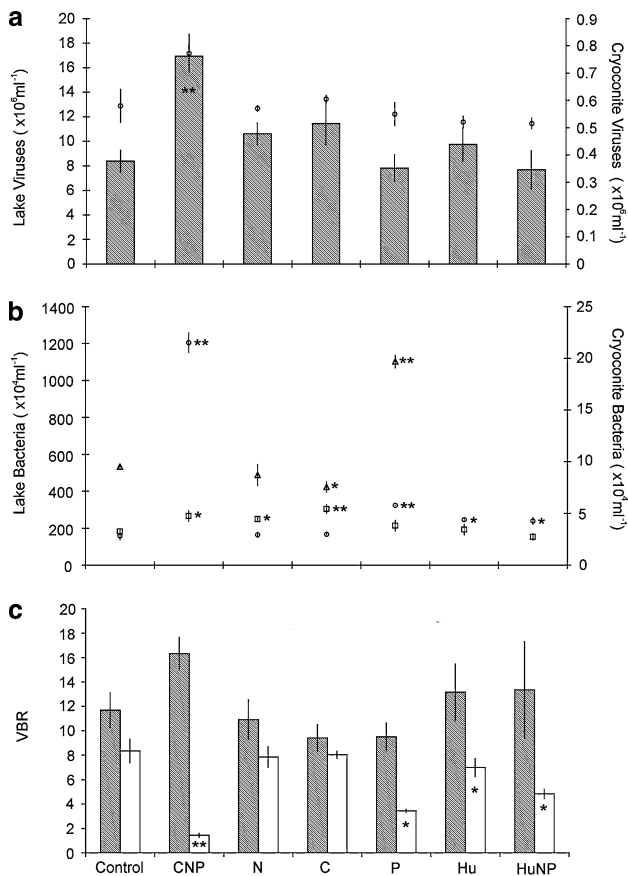


Fig. 3 **a** Mean concentration of viruses, where striped columns represent cryocoinite treatment at 0.2°C and circles lake treatment at 12°C. **b** Mean concentration of bacteria, where circles represent lake treatment at 12°C, squares cryocoinite treatment at 0.2°C and triangles cryocoinite treatment at 12°C. **c** Mean virus to bacteria ratio (VBR), where striped columns represent cryocoinite treatment at 0.2°C and open columns lake treatment at 12°C. Treatments are no amendment (control), glucose and nutrient addition (CNP), humic substance and nutrient addition (HuNP) only glucose addition (C), phosphorus addition (P), or nitrogen addition (N)

cryocoinite nutrient amended treatment at 12°C there was significantly shorter doubling times in the P treatment relative to the control ($P < 0.01$, one-way ANOVA). In the lake water experiment, all nutrient amended treatments (except the C and N treatment) had significantly shorter doubling times relative to the control (Fig. 4).

The effect of temperature on doubling times was tested with an independent sample T-test. There was a significant reduction in the cryocoinite bacterial doubling time, in all the nutrient amended treatments and the control, when the water temperature increased from 0.2 to 12°C ($P < 0.05$) (Fig. 4). An increase from 0.2 to 4°C in the cryocoinite treatments resulted in a significant reduction in doubling times in the CNP and P treatments ($P < 0.01$ and $P < 0.05$, respectively). The shortest doubling times were noted in the lake water treatments at 12°C (Fig. 4), furthermore, significantly shorter doubling times were noted in the lake water

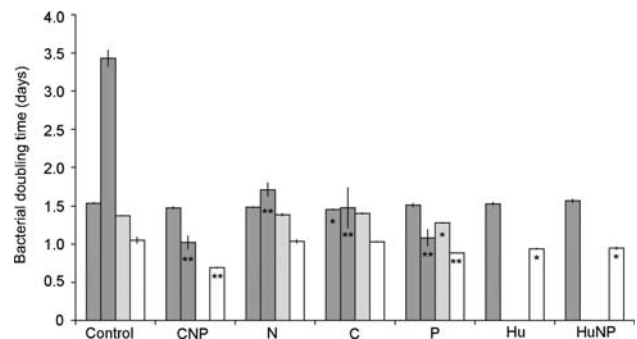


Fig. 4 Mean bacterial doubling times from the 2002 and 2003 nutrient amended experiments, where striped columns represent cryocoinite treatment at 0.2°C, dark grey columns cryocoinite treatment at 4°C, light grey columns cryocoinite treatment 12°C and open columns lake treatment 12°C. Error bars represent \pm SE, $N = 3$. Treatment significantly different to the control is denoted by either * $P < 0.05$ or ** $P < 0.01$

treatments when compared to the cryocoinite treatments (0.2, 4 and 12°C) ($P < 0.01$).

Discussion

Field survey

All the Arctic freshwater environments in the present study can be classed as ultra-oligotrophic to oligotrophic based on chlorophyll *a*, DOC and DN concentrations. Bacterial and viral concentrations and bacterial production were low, but similar to previous values recorded in polar lakes, ponds and cryocoinite holes as well as oligotrophic temperate lakes (e.g. Laybourn-Parry et al. 2001, 2004; Granéli et al. 2004a; Säwström et al. 2002; Hofer and Sommaruga 2001).

In general, viral numbers are higher in nutrient-enriched environments and often positively correlated with bacterial numbers (Wommack and Colwell 2000; Weinbauer 2004). This appeared to be the case in the Arctic aquatic environments that we investigated as bacterial concentration was positively correlated with both virus and DOC concentrations. High virus concentrations were found in Ny-London lake and a small meltwater pool which also had high bacterial numbers and DOC. These habitats were substantially more productive than the other aquatic environments we investigated as they received high inputs of both bird and fox faeces (personal observation). Lowest viral concentrations were associated with the glacial ecosystem (supra- and subglacial streams and the cryocoinite hole).

Viral concentrations exceeded bacterial concentrations in all the investigated Arctic freshwaters resulting in an average virus-to-bacterium ratio of 13.3. The average VBR value in the Arctic field study fall within the bracket of

reported VBR values (range 1–34) from Antarctic freshwaters (Lisle and Priscu 2004; Kepner et al. 1998; Sävström et al. 2007, Laybourn-Parry et al. 2001). It has previously been suggested that VBR values >10 indicate that viral infection might be responsible for a large fraction of bacterial mortality or that viruses are able to persist longer than bacteria (Wommack and Colwell 2000, Yager et al. 2001). Five out of the eight investigated Arctic freshwater environments had VBR values >10 thus suggesting that viral-mediated bacterial mortality may be significant in some Arctic lakes and glacial ecosystems.

The lack of a significant increase in viral numbers after Mitomycin C addition indicates that lysogeny was of little importance in the investigated Arctic freshwater environments. Studies from Antarctic lakes occasionally show high rates of lysogeny, for instance 62.5% of the bacteria were lysogenic in the lakes of McMurdo Dry Valleys (Lisle and Priscu 2004). Annual lake-studies in the Antarctic have shown seasonal variation in lysogeny, with high rates noted in the winter and spring (Laybourn-Parry et al. 2006; Sävström et al. 2007). The use of Mitomycin C as an inducing agent of lysogenic bacteria in freshwaters may be questioned, as the susceptibility of natural bacterioplankton communities to Mitomycin C is still undetermined (Wommack and Colwell 2000). Thus, it is possible that only a part of the lysogenic bacterial population was affected in our samples, thereby resulting in an underestimation of the occurrence of lysogens in the investigated environments. It is also possible that the lack of lysogeny detected in the field study could have been attributed to the time of the year the study was conducted. Previous studies in Antarctic lakes have shown larger proportion of lysogenic bacteria in the winter period rather than in the summer (Sävström et al. 2007; Laybourn-Parry et al. 2006)

Nutrient and temperature manipulation experiments

Nutrient effect

Our results imply that bacterial growth was primarily limited by phosphorus and organic carbon acting as an important secondary limiting nutrient. In the 2002 cryoconite nutrient experiment, the initial lag (between 0 and 72 h) in bacterial growth indicates a delay in the assimilation of nutrients into new bacterial biomass as new enzymes were probably required for processing the additional nutrients. Another possibility is a bacterial community shift occurring in the bottles.

During the time-course of our experiment we failed to detect any significant increase in viral concentrations with the exception of one treatment. In the cryoconite CNP treatment, viral concentrations doubled when compared to the control, which also coincided with a significant increase in

bacterial numbers. The addition of P alone or in combination with humic substances showed no significant changes in viral numbers in either lake or cryoconite water. The general lack of response to nutrient addition in regards to viruses could have several explanations: (1) low initial viral concentrations resulting in slow or none viral production due to reduced host encounter; (2) viral loss processes were high due to the addition of nutrients, this could particularly be the case in the humic treatments as humic substances may influence viruses negatively (Anesio et al. 2004); (3) an initial lag in bacterial growth response to the nutrient additions resulted in a delayed response in viral production, which the time-course of the experiment failed to capture; (4) it is also possible that not all host/virus combinations from the original sample were present in the experiment set up due to the viral loss during filtration or exclusion of specific host bacteria. The lowered VBR values (as noted in the lake water treatment) further suggest that the bacteria increased due to the nutrient enhancement proliferated without the pressure of viral infection. This in turn could indicate that a phage-resistant bacterial community was developing.

Temperature effect

The impact of temperature on bacterial production and growth has been extensively discussed and several studies have found that there is a strong positive relationship between growth rates and temperature in natural bacterioplankton assemblages (e.g. Wiebe et al. 1992; Panzenböck et al. 2000; White et al. 1991; Shiah and Ducklow 1994). Furthermore, bacterioplankton growth at low temperatures (−1.5 to 4°C) is often, but not always, associated with an enhanced substrate requirement (Wiebe et al. 1992; Yager and Deming 1999; Reay et al. 1999; Nedwell 1999). The active uptake of substrates at low temperatures is significantly reduced due to decreasing cell membrane fluidity (Nedwell 1999). However, an increase in substrate concentrations can overcome the negative effect of low temperature on both mesophilic and psychrotolerant bacteria (Wiebe et al. 1992). Arctic freshwater lakes may experience water temperatures of up to 15°C during the summer period whereas cryoconite holes have stable and low temperatures around 0.1°C (Sävström et al. 2002). In our study we raised the cryoconite water temperature to 4 and 12°C and this resulted in an increase in bacterial concentrations and significantly shorter bacterial doubling times. We suggest that the apparent stimulation of bacterial growth at higher temperatures was related to improved uptake of nutrients due to changed membrane properties (Nedwell 1999; Reay et al. 1999). Hence, our results seem to follow the general pattern that bacterial growth responds more quickly and to a greater extent to nutrient amendments at

higher temperatures (e.g. Kirchman and Rich 1997; Granéli et al. 2004b; Nedwell 1999).

In conclusion, our field data showed a strong bacteria–virus coupling and VBR values in general were over 10 which suggests that viral-mediated bacterial mortality may be significant in Arctic lake and glacial ecosystems. Lysogeny was not detected in any of the investigated environments thus indicating that the lysogenic pathway was of minor importance at the sampling time. Our experimental results indicated that multiple limiting factors were involved in controlling bacterial growth. Bacterioplankton growth was co-limited by phosphorus and possibly organic carbon and was also stimulated by increasing temperatures. However, only the cryoconite CNP-treatment induced increases in both virus and bacteria concentrations. Even though an increase in phosphorus concentration had a positive effect on bacteria this was not reflected in the virus concentration. This indicates that viral proliferation was not stimulated by the same parameters that controlled bacterial growth in our experiment.

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