

Experimental evaluation of the warming effect on viral, bacterial and protistan communities in two contrasting Arctic systems

Elena Lara^{1,*}, Jesús M. Arrieta², Iñigo García-Zarandona², Julia A. Boras¹, Carlos M. Duarte^{2,3}, Susana Agustí^{2,3,4}, Paul F. Wassmann⁵, Dolors Vaqué¹

¹Departament de Biologia Marina i Oceanografia, Institut de Ciències del Mar (CSIC), Passeig Marítim de la Barceloneta 37–49, 08003 Barcelona, Spain

²Global Change Research Department, Institut Mediterráni d' Estudis Avançats (CSIC-UIB), Miquel Marquès 21, 07190 Esporles, Illes Balears, Spain

³The UWA Oceans Institute, and ⁴School of Plant Biology, University of Western Australia, 35 Stirling Highway, 6009 Crawley, Western Australia, Australia

⁵Department of Arctic and Marine Biology (AMB), Faculty of Biosciences, Fisheries and Economics, University of Tromsø, 9037 Tromsø, Norway

ABSTRACT: The effect of Arctic warming, which is 3 times faster than the global average, on microbial communities was evaluated experimentally to determine how increasing temperatures affect bacterial and viral abundance and production, protist community composition, and bacterial loss rates (bacterivory and lysis) in 2 contrasting Arctic marine systems. In July 2009, we collected samples from open Arctic waters in the Barents Sea and Atlantic-influenced waters in Isfjorden, Svalbard Islands (Fjord waters). The samples were used in 2 microcosm experiments at 7 temperatures, ranging from 1.0 to 10.0°C. In the open Arctic microbial community, collected at <1.0°C, bacterial and viral abundances, bacterial production and grazing rates due to protists increased significantly above 5.5°C, and remained at high values at even higher experimental temperatures. The abundance of protists, such as some heterotrophic pico/nanoflagellates, as well as some ciliates, also increased with warming. In contrast, the biomass of phototrophs decreased above 5.5°C. The water temperature in Fjord waters was 6.2°C at the time of sampling, and the microbial community showed smaller variations than the Arctic community. These results indicate that increases in temperature stimulate heterotrophic microbial biomass and activity compared to that of phototrophs, which has important implications for carbon and nutrient cycling in the system. In addition, open Arctic communities were more vulnerable to warming than those already adapted to the warmer Fjord waters influenced by Atlantic seawater.

KEY WORDS: Virus · Bacteria · Protists · Viral production · Grazing · Global warming

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INTRODUCTION

The Earth's climate is changing and global temperatures are rising at unprecedented rates (IPCC 2007). Warming is particularly intense in the Arctic Ocean, where temperatures are increasing at rates of 0.4°C per decade (ACIA 2004). Moreover, this rise is ex-

pected to continue to accelerate, resulting in a 9°C increase over the 21st century (IPCC 2007). The consequences of these increasing temperatures are already visible in the Arctic; for example, the loss of ice cover is now affecting the habitat of large mammals, birds and humans (Smetacek & Nicol 2005, Wassmann et al. 2011), and extensive sea ice melting has led to

*Email: elara@icm.csic.es

large changes in the biogeochemistry (Chen et al. 2003, Wassmann et al. 2011) and functioning of microbial food webs in Arctic waters (Boras et al. 2010).

The microbial loop is fundamental to the functioning of Arctic marine ecosystems (Nielsen & Hansen 1995, Iversen & Seuthe 2011) in which the bacterioplankton play a pivotal role as recyclers of the available nutrients (Thingstad & Martinussen 1991, Seuthe et al. 2011). However, viral lysis of bacterioplankton interrupts this cycle and converts the particulate organic matter back into dissolved organic matter, which then becomes available again to other bacteria (Fuhrman 1999). Temperature is a potentially limiting factor that affects biogeochemical processes (Nedwell 1999): microbial growth, respiratory rates and organic carbon assimilation are all affected by changes in water temperature (Holding et al. 2013). Hence, it is extremely important to understand the effect of warming on microbial communities, given the central role they play in the ocean carbon cycle. Although Li & Dickie (1987) found that photosynthetic activity was much greater than heterotrophic activity in a study of seasonal variations, recent studies show that warming stimulates the respiration of plankton communities faster than their photosynthetic rates (Harris et al. 2006, López-Urrutia et al. 2006, Regaudie-De-Gioux & Duarte 2012). Moreover, an increase in temperature favors smaller organisms in aquatic systems for a given level of resources (Daufresne et al. 2009), leading to a higher proportion of picophytoplankton among the autotrophs (Morán et al. 2010) and increasing heterotrophic bacterial activity (Iribarri et al. 1985, White et al. 1991). Protist grazing and viral activity are closely linked with bacterial abundance (BA) and activity, so any change in the bacterial metabolic state, abundance or distribution would affect these processes. It has been well established that bacterial losses due to protist grazing increase with temperature. Thus, Vaqué et al. (2009) found an increase in bacterial production (BP) and ingestion rates after a certain experimental temperature was reached at different Antarctic sites, and Boras et al. (2010) showed that protist grazing dominates in surface waters of the Arctic Ocean that receive ice-melt waters. Increases in temperature are also likely to influence the interactions between viruses and the cells they infect. If prokaryotic growth rates increase with temperature, the length of the lytic cycle decreases and the burst size (BS) increases, thus increasing viral production (VP) (Proctor et al. 1993, Danovaro et al. 2011). The viral life strategy in the oceans (lytic and lysogeny) also depends largely on the physiological state of the host

and the physico-chemical conditions of the environment (Miller 2001). The environmental factors that influence the adoption of a lysogenic strategy are currently not well understood, and the molecular mechanisms that govern whether a phage enters a lysogenic or lytic cycle are still unclear (Long et al. 2008). Understanding these aspects could help to clarify the effects of changing environmental conditions induced by climate change. Due to the prevailing conditions of limited nutrients, periods of low host abundance and production and low temperatures, lysogeny could be expected to be a common phenomenon in the Arctic Ocean (Angly et al. 2006). Nevertheless, the potential effects of temperature on the viral life strategy are still unclear.

In the present study, we experimentally tested how autotrophic and heterotrophic Arctic microbial communities responded to various temperatures, based on the predicted warming of the sea surface temperature in the Arctic Ocean. In particular, we investigated changes in phytoplankton biomass, microbial abundances, flagellate community size structure, ciliate community composition, bacterial and viral production, and losses due to bacterivory and viral lysis. We also identified temperatures where further warming triggered a significant shift for each of the studied variables. For this purpose, we used temperature-controlled microcosms with seawater collected from 2 contrasting Arctic ecosystems: one located in open Arctic waters and one in Fjord waters.

MATERIALS AND METHODS

Study area and sampling

Two consecutive 10-d microcosm experiments were carried out in summer 2009 at the UNIS (University of Svalbard) facilities in Longyearbean (Svalbard Islands, Norway). Seawater for the microcosm experiments was collected at 2 different Arctic locations. The first sampling point was located in the Barents Sea, southeast of Svalbard, Norway ($76^{\circ} 28' 65''$ N, $28^{\circ} 00' 62''$ E) (Fig. 1). Water was sampled on 28 June 2009 at 26 m depth (surface waters) on board the RV 'Jan Mayen' using a CTD rosette sampler. Arctic water (840 l , $T = -1.19^{\circ}\text{C}$, salinity 33.92) was collected and distributed in 14 polypropylene containers (60 l) previously treated with HCl 0.1N for at least 48 h and thoroughly rinsed with seawater from the sampling site. Seawater containers were stored in the dark at 0°C for 48 h in a controlled temperature room on board and at UNIS (for logistical reasons) until the

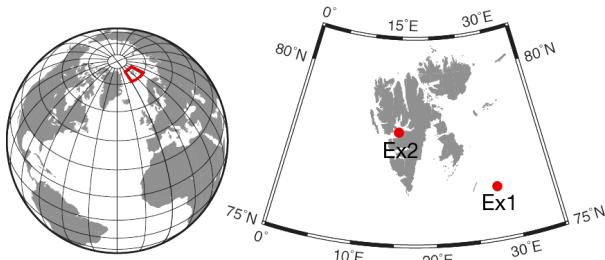


Fig. 1. Sampling locations of the 2 microcosm experiments. Ex1: first sampling point located in the Barents Sea (open Arctic waters). Ex2: second sampling point located at Isfjorden (Svalbard Islands)

start of the experiment. The second sampling point was located at Isfjorden, the second largest fjord in Svalbard Island, Norway ($78^{\circ}20'00''\text{N}$, $15^{\circ}00'00''\text{E}$) ($T = 6.2^{\circ}\text{C}$, salinity 32.73). On 8 July 2009, 840 l of fjord water was sampled (from on board a rubber boat) from 2 m depth using a peristaltic pump. The collected water was distributed and stored in the experimental carboys, as described for the first sampling point. The water samples were transported to the UNIS facilities, and the experimental treatment started immediately upon arrival.

Experimental set-up

Seawater samples from different carboys (60 l) were mixed together in larger containers (280 l) filtered through a 150 µm mesh net to remove larger grazers and transferred to 14 acid-cleaned polycarbonate carboys (microcosms, 20 l). Duplicate carboys for each experimental temperature were submerged in 7 tanks (280 l) connected to a temperature control unit (PolyScience 9600 series) with an impelling and expelling pump. Seven experimental temperatures, ranging from 1.0 to 10.0°C increasing in 1.5°C steps, were tested (Fig. 2A). Temperature data loggers submerged in each tank were used to monitor the resulting water temperature. The experimental set-up was completed with 2 fluorescent light tubes per tank to provide the appropriate light. The light emitted from fluorescent lamps was 90 µmol photons $\text{m}^{-2} \text{s}^{-1}$ (measured using a LI-1000 Li-Cor radiation sensor). This irradiance was selected so as to reproduce a light environment similar to where the plankton communities were collected, based on measurements from earlier cruises in the same season. For the open Arctic community samples, the temperature was increased gradually over the first 3 d of the experiment from 1.0°C to each final treatment temperature (Fig. 2A). For the Fjord community, the temperature

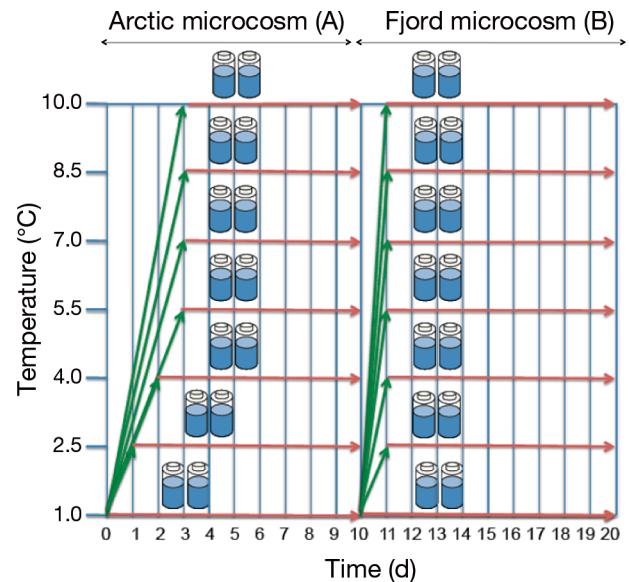


Fig. 2. Schematic representation of 2 experimental microcosms used to study the effects of Arctic warming on microbial communities. Duplicate carboys were incubated at 7 experimental temperatures, ranging from 1.0° to 10.0°C increasing in 1.5°C steps. (A) Arctic microcosms: the temperature was increased gradually over the first 3 d of the experiment from 1.0°C to each final treatment temperature. (B) Fjord microcosms: the temperature was immediately set to each final treatment temperature

was set immediately to each final treatment temperature because the temperature of the original water was close to the middle of the experimental temperature range (Fig. 2B). Unfortunately, the treatment at 7.0°C had to be discarded after a malfunction in the cooling system that caused a sustained temperature increase to well above the experimental temperature range during the first 3 d of the experiment.

Chlorophyll a concentration

Daily subsamples (50 ml) from each carboy were filtered through Whatmann GF/F glass-fiber filters. After filtration the pigment was extracted in 90% acetone for 24 h and kept refrigerated in the dark. Filters were analyzed according to the fluorometric method of Parsons et al. (1984) and fluorescence was measured spectrophotometrically.

Microbial abundances

Samples for viral abundance (VA) and bacterial abundance (BA) were collected daily from each microcosm, while pico/nanoflagellate and ciliate

abundances were determined once every 2 d for each experimental temperature over the entire experimental period. Subsamples (2 ml) for VA were fixed with glutaraldehyde (0.5 % final concentration), refrigerated, quick frozen in liquid nitrogen and stored at -80°C, as described in Marie et al. (1999). Counts were made using a FACSCalibur flow cytometer (Becton and Dickinson) with a blue laser emitting at 488 nm. Samples were stained with SYBR Green I and run at an optimal event rate (between 100 and 800 events s⁻¹) (Marie et al. 1999), which in our cytometer corresponded to the medium flow speed (Brussaard 2004). Samples (50 ml) were fixed with glutaraldehyde (1 % final concentration) for bacteria and pico/nanoflagellate (\leq 2 to 20 μm) counts. Subsamples of 10 ml for bacteria and 20 ml for pico/nanoflagellate abundances were filtered through 0.2 and 0.6 μm black polycarbonate filters respectively, and stained with DAPI (4,6-diamidino-2-phenylindole) (Porter & Feig 1980) to a final concentration of 5 $\mu\text{g ml}^{-1}$ (Sieracki et al. 1985). The abundances of these microorganisms were determined by epifluorescence microscopy (Olympus BX40-102/E, at 1000 \times). Between 200 and 300 bacteria were counted per sample and at least 50 to 300 heterotrophic or phototrophic pico/nanoflagellates were counted per filter from 3 to 4 transects of 5 to 10 mm each. They were grouped into 3 size classes: \leq 2 μm , 2–5 μm , $>$ 5 μm . Pico and nanoflagellates showing red-orange fluorescence and/or plastidic structures in blue light (B2 filter) were considered phototrophic pico/nanoflagellates (PF), while colorless flagellates showing yellow fluorescence were counted as heterotrophic pico/nanoflagellates (HF). With this method, we could not distinguish mixotrophic flagellates. The abundances of ciliates and the phagotrophic dinoflagellate *Gyrodinium* sp. were obtained using the Utermöhl method. 125 ml of sample was fixed with acidic lugol (2 % final concentration). Aliquots of the fixed samples (50 to 100 ml) were settled for 24 to 48 h before enumeration. Both the ciliates and the dinoflagellate *Gyrodinium* sp. were counted in an inverted microscope (Zeiss AXIO-VERT35, at 400 \times). Up to 200 ciliates and 100 *Gyrodinium* sp. were counted per sample. Ciliates were identified to genus level when possible (Lynn & Small 2000), and were grouped into the subclasses Oligotrichia: oligotrichs (*Halteria* sp., *Strombidium* sp. and *Laboea* sp.); Choreotrichia: naked choreotrichs (*Strobilidium* sp.) and loricate choreotrichs (tintinnids); Haptoria: haptorids (*Myrionecta* sp. and *Askenasia* sp.); Scuticociliatida (Scuticociliates); and Hypotrichia (*Euploites* sp.).

Bacterial production

Bacterial production (BP) was measured by incorporation of radioactive ³H-leucine following Kirchman et al. (1985) and modified by Smith et al. (1992). Aliquots of 1.5 ml were taken at time zero from *in situ* and every day from each microcosm and were dispensed into 4 vials (2 ml) plus 2 TCA-killed control vials. Next, 48 μl of a 1 μM solution of ³H-leucine was added to the vials to obtain a final concentration of 40 nM. Incubations were run for 2 to 3 h in the same thermostatic chambers as the experimental microcosms, and stopped with TCA (50 % final concentration). Tubes were then centrifuged for 10 min at 12 000 \times g. Pellets were rinsed with 1.5 ml of 5 % TCA, stirred and centrifuged again. Supernatant was removed and 0.5 ml of scintillation cocktail was added. The vials were counted in a Beckman scintillation counter. For each time point, BP was expressed in $\mu\text{mol C l}^{-1} \text{d}^{-1}$ of ³H-leucine incorporation, applying a conversion factor of 1.5 kg C mol Leu⁻¹ (Kirchman 1992).

Viral production and bacterial losses

Samples for determining viral production (VP) and bacterial mortality due to protists (PMM) and viruses (VMM) in the Arctic community were taken 3 times: at time zero (-1°C), and on Days 4 and 8 of the experiment, for all experimental temperatures. For the Fjord community, samples were taken twice: at time zero (5.5 °C) and on Day 8 for 4 experimental temperatures (1.0, 5.5, 8.5 and 10.0°C). So that there would be enough water volume to measure the viral production and viral lysis, 0.5 l subsamples from each experimental duplicate were pooled together. PMM was evaluated following the fluorescent-labeled bacteria (FLB) disappearance method (Sherr et al. 1987, Vázquez-Domínguez et al. 1999). For each measurement of the grazing rates, duplicated 1.5 l sterile bottles were filled with 0.5 l aliquots of seawater from each experimental microcosm, and a third bottle was filled with 0.5 l of grazer-free water as a control. Each duplicate and control was inoculated with FLB at 20 % of the natural bacterial concentration. The FLB were prepared with a culture of *Brevundimonas diminuta* (<http://www.cect.org>) as described in Vázquez-Domínguez et al. (1999). Bottles were incubated in the tanks at the same experimental temperature as the corresponding microcosms and in the dark for 48 h. Samples for evaluating the pico/nanoflagellate abundances were taken at the initial time of the

grazing assay. To assess the bacterial and FLB abundances, samples were taken at the beginning and at the end of the grazing assay. Abundances of bacteria, FLB and pico/nanoflagellates were assessed by epifluorescence microscopy as explained above. Natural bacteria were identified by their blue fluorescence when excited with UV radiation, while FLB were identified by their yellow-green fluorescence when excited with blue light. Control bottles showed no decrease in FLB at the end of the incubation time. The grazing rates of bacteria were obtained according to the equations of Salat & Marrasé (1994), based on the specific grazing rate (g) and the specific net growth rate (a), and calculated as follows:

$$g = -(1/t) \ln (\text{FLB}_t/\text{FLB}_0) \quad (1)$$

$$a = (1/t) \ln (\text{BA}_t/\text{BA}_0) \quad (2)$$

where t is the incubation time, FLB_t is the abundance of FLB at the final time, FLB_0 is the abundance of FLB at the initial time, and BA_t and BA_0 are bacterial abundances at the end and beginning of the incubation time, respectively.

The net bacterial production (BPN, cells $\text{ml}^{-1} \text{d}^{-1}$) in the incubation bottles was obtained with the equation:

$$\text{BPN} = \text{BA}_0 \times (e^{at}) \quad (3)$$

Then, the grazing rate (G , cells $\text{ml}^{-1} \text{d}^{-1}$) was calculated as:

$$G = (g/a) \times \text{BPN} \quad (4)$$

Finally, PMM as the percentage of the bacterial standing stock (PMM_{BSS} , % d^{-1}) was calculated as:

$$\text{PMM}_{\text{BSS}} = (G \times 100)/\text{BA}_0 \quad (5)$$

We used the virus-reduction approach to determine the VP, as well as bacterial losses due to phages (Wilhelm et al. 2002). Briefly, 1 l of seawater from each experimental microcosm was pre-filtered through a 0.8 μm pore sized cellulose filter (Whatman) and then concentrated by a spiral-wound cartridge (0.22 μm pore size, VIVAFLOW200) to obtain 50 ml of bacterial concentrate. Virus-free water was collected by filtering 0.5 l of seawater using a cartridge with a 30 kDa molecular mass cutoff (VIVAFLOW200). A mixture of virus-free water (150 ml) and bacterial concentrate (50 ml) was prepared and distributed into 4 sterile 50 ml Falcon plastic tubes. Two of the tubes were kept as controls, and mitomycin C (Sigma) was added (1 $\mu\text{g ml}^{-1}$ final concentration) to the other 2 tubes as the inducing agent of the lytic cycle. All Falcon tubes were incubated in the tanks at the same temperature as the microcosms and in the dark for 12 h. Samples for VA and BA were

collected at time zero and every 4 h of the incubation, fixed with glutaraldehyde (0.5 % final concentration) and stored as described above. Virus and bacterial numbers from the VP incubations were counted by flow cytometry. The number of viruses released by bacterial cells (burst size) was estimated from VP measurements, as in Middelboe & Lyck (2002) and Wells & Deming (2006). The increase in VA over short time intervals (4 h) in VP incubations was divided by the decrease in BA in the same time period. We assumed that BP and viral decay in this time interval were negligible. We estimated BS to range from 11 to 82 viruses per bacterium. VMM was determined as previously described in Weinbauer et al. (2002) and Winter et al. (2004). Briefly, an increase in VA in the control falcon tubes represents lytic viral production (VP_L), and the difference between the viral increase in the mitomycin C treatments and VP_L gives the lysogenic production (VP_{LG}). Because part of the bacteria is lost during the bacterial concentration process, VP_L and VP_{LG} were multiplied by the bacterial correction factor to compare the VP values from different incubations. This factor was calculated by dividing the *in situ* bacterial concentrations by the time zero bacterial abundances in the VP measurements (Winget et al. 2005) and in our case ranged between 0.75 and 2.15. We then calculated the rate of lysed cells (RLC, cells $\text{ml}^{-1} \text{d}^{-1}$) by dividing VP_L by BS, as described in Guixa-Boixereu (1997). RLC was used to calculate VMM as a percentage of the bacterial standing stock (VMM_{BSS} , % d^{-1}):

$$\text{VMM}_{\text{BSS}} = (\text{RLC}_{\text{GR}} \times 100)/\text{BA}_0 \quad (6)$$

where BA_0 is the initial bacterial abundance in the viral production incubation tube. Assuming that the percentage of BSS losses due to viruses is the same in the falcon tubes and grazing bottles, we used VMM_{BSS} to calculate the rate of lysed bacteria in the grazing bottles (RLC_{GR} , cells $\text{ml}^{-1} \text{d}^{-1}$):

$$\text{RLC}_{\text{GR}} = (\text{VMM}_{\text{BSS}} \times \text{BA}_{\text{GR}})/100 \quad (7)$$

where BA_{GR} is the bacterial abundance in the grazing bottles at time zero.

Statistical analysis

The Shapiro-Wilk W -test was used to check the normal distribution of the data, and data were logarithmically transformed prior to analyses if necessary. 1-way ANOVA was used to detect a significant shift between 2 consecutive increasing temperatures for each of the variables studied. This means that the

comparisons of all data (for each variable) before and after the shift were statistically significant. These statistical analyses were performed using the Kaleidagraph V4.0 and JMP programs. For each experimental temperature, we calculated the average of each variable \pm SE for the whole experimental period in the Arctic and Fjord microcosms.

RESULTS

Physical and biological variables in Arctic and Fjord waters

The 2 environments showed clear differences at the time of sampling (Table 1). The water temperature was lower in the open Arctic waters (-1.2°C) than in the Fjord waters (6.2°C). Pigmented microorganism (flagellates and ciliates, e.g. *Myrionecta* sp.) abundances, as well as chlorophyll *a* (chl *a*) concentrations were higher in the open sea Arctic community. In contrast, most heterotrophic variables, such

Table 1. *In situ* values of temperature, chlorophyll and microbiological variables for Arctic and Fjord waters. Chl *a*: chlorophyll *a*; PF: phototrophic pico/nanoflagellates, and the 2 main identified genera: *Micromonas* sp. ($\leq 2 \mu\text{m}$ size class) and *Phaeocystis* sp. ($2-5 \mu\text{m}$); BA: bacterial abundance; VA: viral abundance; HF: heterotrophic pico/nanoflagellates, and *Gyrodinium* sp. (phagotrophic dinoflagellate $\geq 30 \mu\text{m}$); BP: bacterial production; VP_L: lytic viral production; VP_{LG}: lysogenic viral production; PMM_{BSS}: protist-mediated mortality as a percentage of bacteria standing stock; VMM_{BSS}: virus-mediated mortality as % of BSS

Variable	Arctic	Fjord
Temperature ($^{\circ}\text{C}$)	-1.2	6.2
Chl <i>a</i> ($\mu\text{g l}^{-1}$)	0.6	0.1
PF ($10^3 \text{ cells ml}^{-1}$)	2.1 ± 0.2	0.8 ± 0.3
<i>Micromonas</i> sp. ($10^3 \text{ cells ml}^{-1}$)	0.4 ± 0.09	0.7 ± 0.2
<i>Phaeocystis</i> sp. ($10^3 \text{ cells ml}^{-1}$)	1.4 ± 0.2	0.02 ± 0.0
BA ($10^5 \text{ cells ml}^{-1}$)	3.8 ± 0.6	8.0 ± 0.6
VA ($10^5 \text{ virus ml}^{-1}$)	5.4 ± 0.0	8.4 ± 1.5
HF ($10^3 \text{ cells ml}^{-1}$)	3.0 ± 0.3	0.4 ± 0.03
HF $\leq 2 \mu\text{m}$ size class ($10^3 \text{ cells ml}^{-1}$)	1.0 ± 0.1	0.02 ± 0.00
HF $2-5 \mu\text{m}$ ($10^3 \text{ cells ml}^{-1}$)	1.2 ± 0.2	0.4 ± 0.05
HF $> 5 \mu\text{m}$ ($10^3 \text{ cells ml}^{-1}$)	0.7 ± 0.0	0.03 ± 0.02
<i>Gyrodinium</i> sp. ($10^3 \text{ cells l}^{-1}$)	0.2 ± 0.0	2.7 ± 0.5
Phagotrophic ciliate ($10^3 \text{ cells l}^{-1}$)	1.4 ± 0.3	1.3 ± 0.0
<i>Myrionecta</i> sp. ($10^3 \text{ cells l}^{-1}$)	3.1 ± 0.9	0.1 ± 0.0
BP ($10^{-2} \mu\text{mol C l}^{-1} \text{ d}^{-1}$)	3.9 ± 0.0	36.4 ± 1.8
VP _L ($10^5 \text{ viruses ml}^{-1} \text{ d}^{-1}$)	1.7 ± 1.4	2.3 ± 0.2
VP _{LG} ($10^5 \text{ viruses ml}^{-1} \text{ d}^{-1}$)	Negligible	3.9 ± 0.0
VMM _{BSS} (% d^{-1})	10.1 ± 6.8	90.6 ± 11.6
PMM _{BSS} (% d^{-1})	15.2 ± 4.7	33.0 ± 24.6

as abundances of bacteria, viruses and *Gyrodinium* sp., as well as BP and VP and bacterial losses, were higher in the Fjord waters, while phagotrophic ciliate abundances were similar in the 2 environments (Table 1). In both systems phototrophic pico/nanoflagellates (PF) were dominated by *Micromonas* sp. (PF $\leq 2 \mu\text{m}$) and free-living forms of *Phaeocystis* sp. (PF $2-5 \mu\text{m}$) (Table 1), while phagotrophic ciliates, such as *Strobilidium* sp. and tintinnids, were the most abundant groups in the Arctic and Fjord waters respectively. Henceforth, the experiments carried out with the open Arctic waters will be called Arctic microcosms and those with water from Isfjorden will be called Fjord microcosms. The microbial communities in the open Arctic waters will be called the Arctic community, and those found in the Isfjorden waters will be called the Fjord community.

Changes in biological variables during the experiments

Chlorophyll *a* concentration and phototrophic pico/nanoflagellate abundance

The minimum and maximum values of the chl *a* concentration for the Arctic and Fjord microcosms over the entire experiment are shown in Table 2. The average chl *a* concentrations at each temperature for the entire experimental period are shown in Fig. 3A,B. In the Arctic community, the chl *a* concentration decreased by 50% between 5.5°C and 7°C (Fig. 3A, Table 3). There was a slight decrease in the Fjord microcosms, but no significant differences were recorded between the chl *a* concentration at lower and higher temperatures (Fig. 3B, Table 3). In both experimental microcosms, *Micromonas* sp. (PF $\leq 2 \mu\text{m}$) was the main contributor to the total PF abundance followed by *Phaeocystis* sp. (PF $2-5 \mu\text{m}$). In both systems, there was a very low abundance of PF $> 5 \mu\text{m}$. Minimum and maximum values during the experiments are shown in Table 2. Average values of *Micromonas* sp. in the Arctic microcosms were almost constant at increasing temperatures up to 5.5°C , at which they reached a peak then decreased again at higher temperatures (Fig. 3C). In the Fjord microcosms, *Micromonas* sp. values remained high between 2.5°C and 8°C . We did not detect important changes in PF abundances (Fig. 3D). In the 2 microcosm experiments, free-living PF ($2-5 \mu\text{m}$), such as *Phaeocystis* sp., and PF $> 5 \mu\text{m}$ showed lower abundances (Fig. 3C,D) than PF $\leq 2 \mu\text{m}$, such as *Micromonas* sp. (Fig. 3C,D).

Table 2. Minimum and maximum values for each variable in the 2 microcosm experiments. T: experimental temperature. See Table 1 legend for explanations of abbreviations of other variables

Variable	Arctic				Fjord							
	Minimum value	T (°C)	Day	Maximum value	T (°C)	Day	Minimum value	T (°C)	Day	Maximum value	T (°C)	Day
Chl <i>a</i> ($\mu\text{g l}^{-1}$)	0.1	10.0	10	1.7	2.5	6	0.2	10.0	1	3.0	5.5	7
PF ($10^4 \text{ cells ml}^{-1}$)	0.06	2.5	4	2.6	5.5	9	0.05	2.5	0	13.1	2.5	7
BA ($10^5 \text{ cells ml}^{-1}$)	1.3	2.5	4	9.9	5.5	7	7.4	5.5	0	20.4	8.5	3
VA ($10^5 \text{ virus ml}^{-1}$)	1.3	2.5	8	13.8	2.5	8	3.6	10.0	9	17.6	4.0	9
HF ($10^3 \text{ cells ml}^{-1}$)	0.5	7.0	5	4.6	5.5	3	0.2	1.0	8	3.3	4.0	5
<i>Gyrodinium</i> sp. ($10^3 \text{ cells l}^{-1}$)	0.02	7.0	8	0.7	10.0	2	0.2	8.5	3	3.2	5.5	0
Total ciliates ($10^3 \text{ cells l}^{-1}$)	0.04	7.0	7	12.0	10.0	2	0.04	2.5	7	1.5	5.5	0
BP ($\mu\text{mol C l}^{-1} \text{ d}^{-1}$)	0.03	7.0	3	1.3	7.0	9	0.06	10.0	9	0.7	5.5	8
VP _L ($10^5 \text{ viruses ml}^{-1} \text{ d}^{-1}$)	Negligible	5.5/7.0	8	9.0	4.0	8	0.6	5.5	8	1.9	10.0	8
VP _{LG} ($10^5 \text{ viruses ml}^{-1} \text{ d}^{-1}$)	Negligible	1.0/2.5/4.0/ 5.5/7.0	0/4/8	3.7	5.5	8	Negligible	1.0/8.5	8	3.9	5.5	0
PMM _{BSS} (% d ⁻¹)	Negligible	5.5/7	8	17.1	7.0	4	0.8	1.0	8	96.6	5.5	0
VMM _{BSS} (% d ⁻¹)	4.1	4.0	4	35.4	8.5	8	3.3	1.0	8	33.0	5.5	0

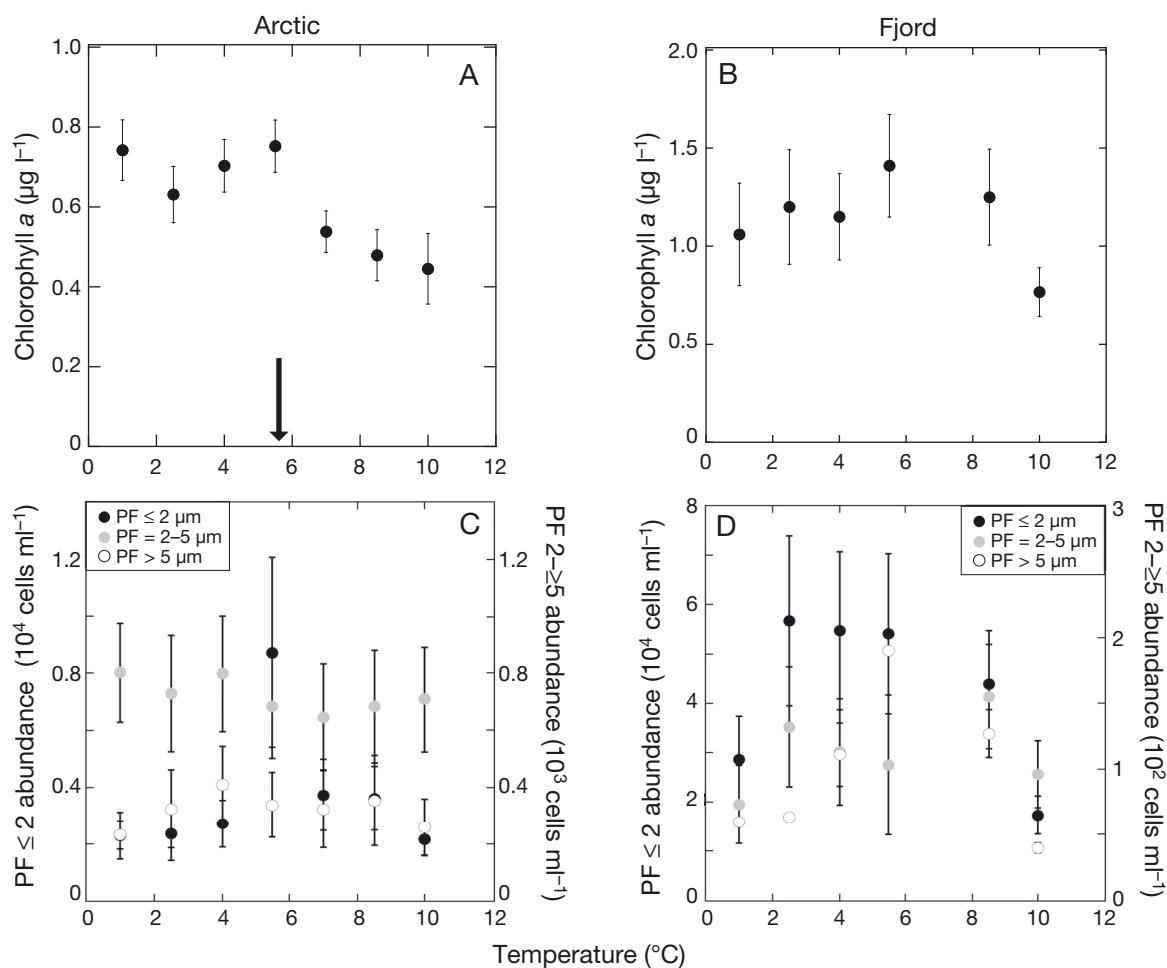


Fig. 3. Average values ($\pm \text{SE}$) over the experimental period for each temperature treatment in Arctic and Fjord microcosms of (A,B) chl *a* concentration and (C,D) abundance of phototrophic pico/nanoflagellates (PF) of different size classes. The arrow in (A) indicates the temperature at which a shift in chl *a* abundance occurred in Arctic samples

Table 3. Temperatures at which significant shifts in measured variables were detected according to 1-way ANOVA. N: sample size; F: F-test of the variance; p: level of significance; T: experimental temperature; ns: not significant. See Table 1 legend for explanations of abbreviations of other variables

Variable	Arctic				Fjord			
	N	F	p	T (°C)	N	F	p	T (°C)
Chl a	169	10.3	0.001	5.5–7.0			ns	–
PF			ns	–			ns	–
BA	112	22.0	<0.0001	4.0–5.5	109	4711.0	<0.0001	2.5–4.0
VA	127	5.8	0.01	4.0–5.5	95	7.2	0.009	5.5–8.5
HF ≤2 µm	43	7.6	0.01	4.0–5.5			ns	–
HF 2–5 µm			ns	–			ns	–
HF >5 µm	54	5.6	0.02	7.0–8.5			ns	–
Total ciliates			ns	–				
Other ciliates			ns	–	52	22.1	0.001	5.5–8.5
BP	71	13.8	0.0004	4.0–5.5	62	3.8	0.05	4.0–5.5
VP _L			ns	–			ns	–
VP _{LG}			ns	–			ns	–
PMM _{BSS}	41	4.7	0.03	5.5–7.0			ns	–
VMM _{BSS}			ns	–			ns	–

Heterotrophic microbial communities

The minimum and maximum values of microbial (bacteria, viruses and protists) abundances when the Arctic and Fjord microcosms were exposed to different temperatures are shown in Table 2. The average BA (bacterial abundance) in the Arctic microcosms increased significantly by around two-fold at temperatures between 4.0 and 5.5°C (Fig. 4A). In the Fjord microcosms the increase in abundances was smaller than for the Arctic microcosms (ca. 1.5 times) and occurred between 2.5 and 4.0°C (Fig. 4B). For both systems, the variations in abundances observed before and after a certain temperature were statistically significant (Table 3). The mean VA (viral abundance) for the Arctic community increased between 4.0 and 5.5°C (Table 3, Fig. 3C) and followed a similar trend to that of BA (Fig. 4A,C). In the Fjord community the average VA dropped significantly between 5.5 and 8.5°C (Table 3, Fig. 4D). Changes in the average HF (heterotrophic pico/nanoflagellates) abundances along the temperature gradients for both microcosm experiments did not show clear patterns (Fig. 4E,F). However, when the different HF size classes in the Arctic microcosms were considered it was found that HF ≤2 µm significantly decreased between 4.0 and 5.5°C, while HF >5 µm increased significantly between 7.0 and 8.5°C (Fig. 4E, Table 3). This was not observed in the Fjord microcosms (Fig. 4F, Table 3). Ciliates and the dinoflagellate *Gyrodinium* sp. did not show clear responses to increasing temperature in either system. Thus, in the Arctic microcosms, we

detected that the pigmented *Myrionecta* sp. was the most abundant ciliate and had a tendency to decrease as the temperature increased (Fig. 4G), while we did not observe changes with temperature for the phagotrophic ciliates *Strobilidium* sp., for the so-called 'other ciliates' (comprising *Strombidium* sp., *Euplotes* sp., *Laboea* sp., tintinnids, scuticociliates, *Askenasia* sp. and *Tontonia* sp.) or for the dinoflagellate *Gyrodinium* sp. (Table 3). In the Fjord microcosms, we found that *Myrionecta* sp., and tintinnids and the dinoflagellate *Gyrodinium* sp. showed lower and higher average values, respectively, than in the Arctic microcosms (Fig. 4G,H), and they did not show any response to warming (Table 3). How-

ever, in the Fjord *Strobilidium* sp. was not always present, and when averaging the abundance for each temperature together with the other identified ciliates we observed that they decreased significantly at the highest temperatures (Fig. 4H, Table 3).

Bacterial and viral production

BP (bacterial production) in the Arctic and Fjord microcosms varied between 0.03 and 1.3 µmol C l⁻¹ d⁻¹ (Table 2). The average BP in the 2 types of microcosms followed the same trend as for bacterial abundance, showing a significant increase between 4.0 and 5.0°C (Fig. 5A,B, Table 3). VP_L (viral lytic production) for the Arctic microcosms ranged between not detectable (at 5.5 and 7.0°C) and 9.0 × 10⁵ viruses ml⁻¹ d⁻¹ at 4.0°C, both on Day 8 (Table 2). VP_{LG} (lysogenic viral production) ranged between not detectable (at several temperatures and on several days) and 3.7 × 10⁵ viruses ml⁻¹ d⁻¹, recorded at 5.5°C on Day 8 (Table 2). Average VP_L was higher than VP_{LG} at all temperatures, except at 5.5°C, when VP_{LG} was 1.5 times higher than VP_L (1.23 × 10⁵ and 1.91 × 10⁴ virus ml⁻¹ d⁻¹ respectively, Fig. 5C), and at 7.0°C, when they were the same (Fig. 5C). In the Fjord microcosms, VP_L showed minimum (0.6 × 10⁵ virus ml⁻¹ d⁻¹) and maximum (1.9 × 10⁵ viruses ml⁻¹ d⁻¹) values on Day 8 at 5.5 and 10.0°C, respectively (Table 2). Lysogeny was not detectable at 1.0 or 8.5°C on Day 8, and the highest value (3.9 × 10⁵ virus ml⁻¹ d⁻¹) was recorded at the beginning of the experiment at 5.5°C (Table 2, Fig. 5D).

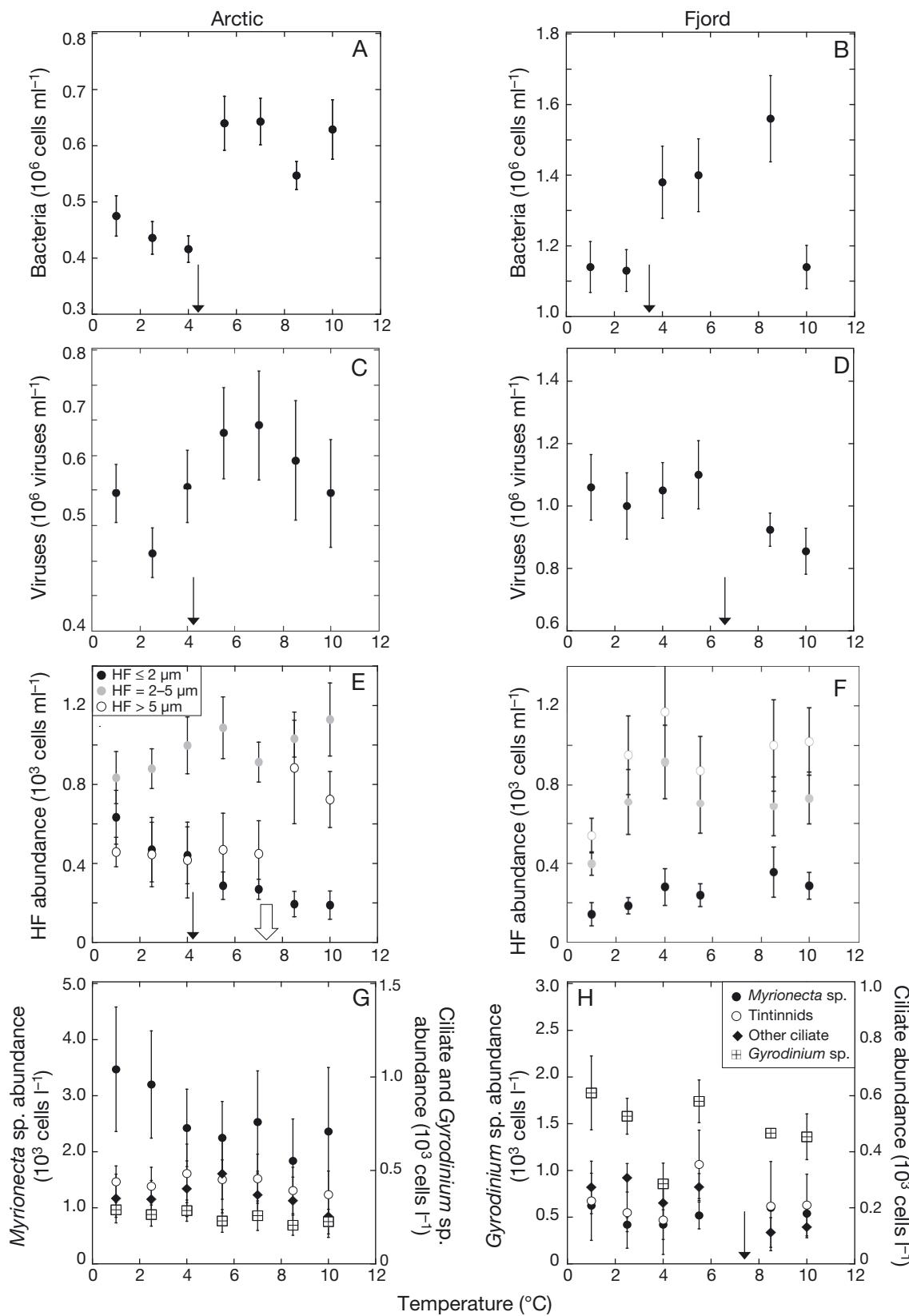


Fig. 4. Average values (\pm SE) over the experimental period in Arctic and Fjord microcosms for each temperature treatment of the abundances of (A,B) bacteria, (C,D) viruses, (E,F) heterotrophic pico/nanoflagellates (HF) and (G,H) ciliates. Arrows indicate the temperatures at which shifts in abundances occurred: in (E) the black arrow marks shifts in abundances of HF $\leq 2 \mu\text{m}$ and the outlined arrow those of HF $> 5 \mu\text{m}$; in (H) the black arrow marks the shifts in 'other ciliates'

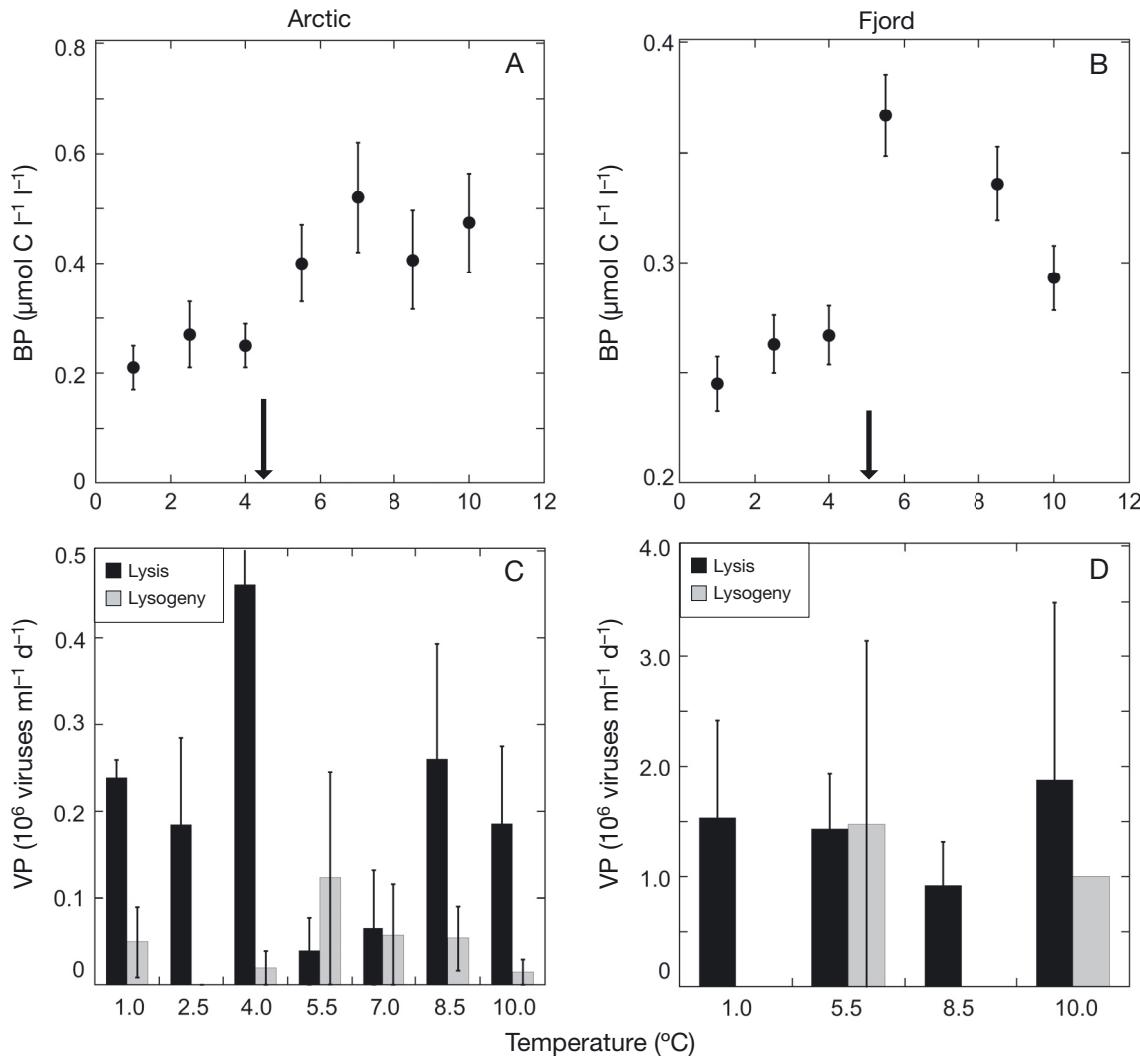


Fig. 5. Average values ($\pm \text{SE}$) over the experimental period of (A,B) bacterial production (BP) for each temperature treatment in Arctic and Fjord microcosms, and average of duplicate subsamples of lytic and lysogenic viral production (VP) for each temperature treatment in (C) Arctic microcosms (measured at Days 0, 4 and 8), and (D) Fjord microcosms (measured at Days 0 and 8). Arrows indicate the temperature at which the shifts in BP occurred

Bacterial mortality

In both experiments, the losses in BSS (bacterial standing stock) due to protists (PMM_{BSS}) were higher than those due to viruses (VMM_{BSS}) in most temperature treatments. In the Arctic microcosms, grazing mostly increased with warming and sampling time above 5.5°C (Fig. 6A,E), showing a significant shift between 5.5 and 7.0°C, except at 10.0°C on Day 8 when values significantly decreased (Table 3, Fig. 6E). In the Fjord microcosms, PMM_{BSS} values recorded on Day 8 increased progressively with increasing temperatures (from 3.3 to 27.0% d^{-1}), but they were always lower than the initial value (Fig. 6B,F).

VMM_{BSS} in the Arctic microcosms reached its maximum value (17.1% d^{-1}) on Day 4 at 7.0°C, and was not detectable at 5.5 and 7.0°C on Day 8 of the experiment (Table 2, Fig. 6C). The average VMM_{BSS} values for each experimental temperature decreased from 1.0 to 5.5°C and increased from 7.0 to 10.0°C. In the Fjord microcosms, the maximum value for VMM_{BSS} was recorded at time zero (96.6% d^{-1}), whereas on Day 8, bacterial lysis increased progressively with the temperature (Fig. 6D). When losses due to protists and viruses were compared in the 2 systems, bacterivory was generally higher than the mortality caused by viruses (Fig. 6E,F), except in the Fjord microcosms at the initial time, when bacterial losses due to viruses were higher than bacterivory (Fig. 6B,D,F).

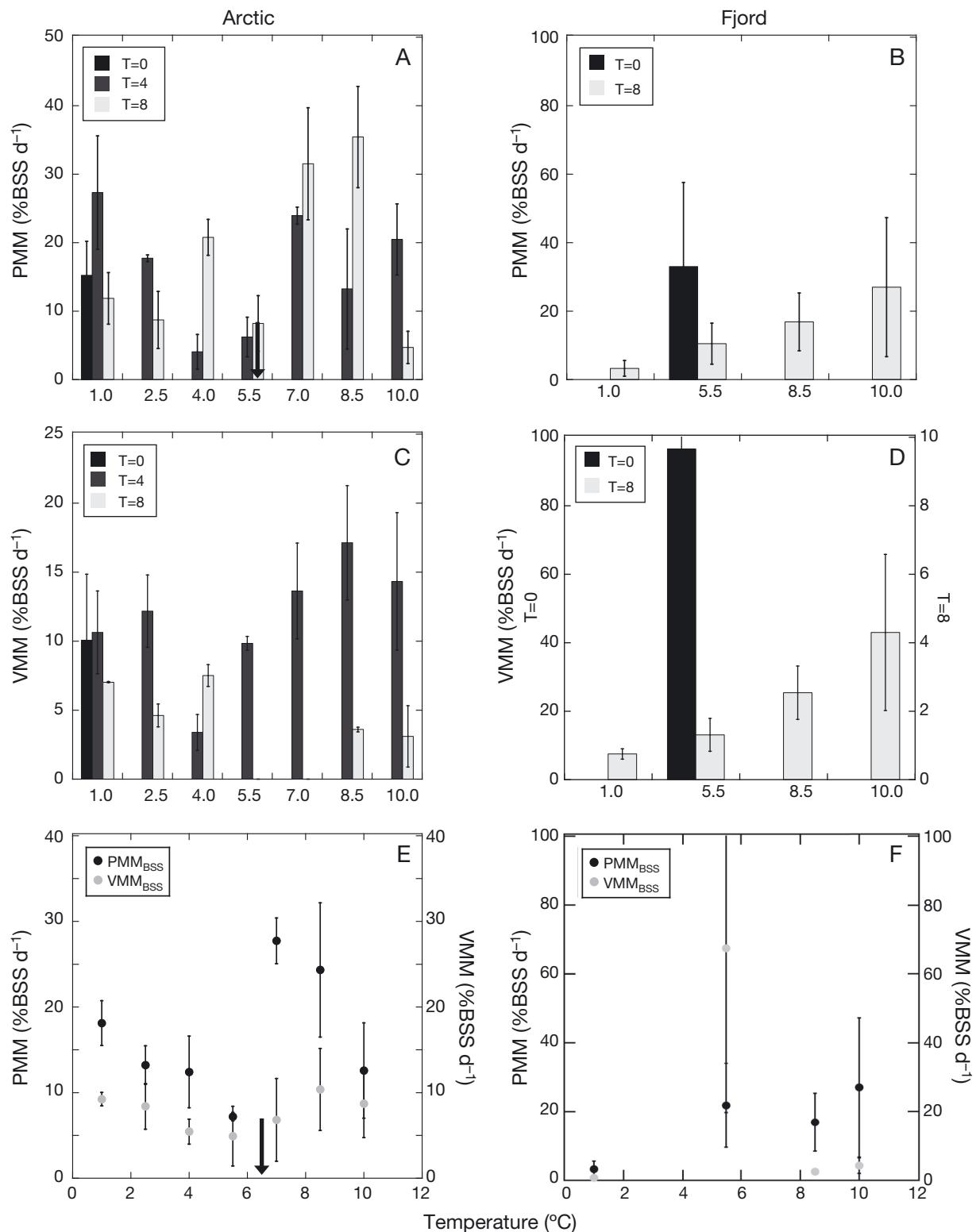


Fig. 6. Average of duplicates of virus-mediated mortality in bacteria as a percentage of the bacterial standing stock (BSS) for each temperature treatment: (A) protist-mediated mortality (PMM_{BSS}) in Arctic microcosms at Days 0, 4 and 8; (B) PMM_{BSS} in Fjord microcosms at Days 0 and 8; (C) virus-mediated mortality (VMM_{BSS}) in Arctic microcosms; (D) VMM_{BSS} in Fjord microcosms. Average values (\pm SE) of PMM_{BSS} and VMM_{BSS} in (E) Arctic and (F) Fjord microcosms for each temperature treatment. Arrows indicate the temperatures at which shifts in bacterial mortality occurred

DISCUSSION

Evaluation of the experimental design used

The natural microbial community was incubated in large microcosms at 7 experimental temperatures, ranging from 1.0 to 10.0°C increasing in 1.5°C steps, to study the variations in abundances as well as growth and mortality rates. Although microcosm experiments could introduce some bias into the development of microbial communities in comparison to natural communities, due to the manipulation of samples and because the reaction of the microorganisms to batch wise incubation occurs in a confined environment, these experimental tools are a useful approach for determining community composition and environmental change rates (Pradeep Ram & Sime-Ngando 2008). Moreover, this effect applies to all experimental microcosms. Hence, the variations in activity and biomass in each microcosm can be compared with each other. In the present study, experimental temperatures were realistic considering the predicted climate change scenarios for the Arctic Ocean over the 21st Century (ACIA 2004). According to the surface satellite temperature (SST), the yearly variations in the temperature at the experimental sampling points were within the range of 6.0 to 7.0°C (http://nomad2.ncep.noaa.gov/ncep_data/). In the open Arctic location the temperature varied between -1.0 and ~6.0°C, while in the Atlantic influenced water (the Fjord system) the temperature ranged from 1.0 to 7.0°C. Therefore, the temperature ranges used in our incubations encompassed the yearly temperature variation range plus a 4.0°C increase. However, the increase in the *in situ* temperature over the Arctic summer is about 0.5°C wk⁻¹, while in our experiments the warming rates were much higher (≥1.5°C d⁻¹). Thus, in nature the adaptation and replacement of microbial communities would take place over a longer time scale, which could not be reproduced in our experimental setup. Although this is a problem that we cannot easily solve, it is important to keep in mind that the experimental approach used does not try to mimic nature, but it does allow us to visualize and understand how warming may affect the Arctic microbial food webs and viral shunt mechanisms, and to detect at which temperature there is a significant shift, i.e. when a significant increase or decrease in a given component of the microbial community begins. These shifts should not necessarily be considered irreversible (Duarte et al. 2012) given the seasonal range of temperatures in these Arctic waters (-1.7 to 7.0°C). For instance, when the Fjord microcosms were subjected

to lower temperatures than the *in situ* temperature (6.0°C), we observed a return to lower bacterial abundances and activities with a similar pattern to that of the Arctic microcosms. In short, the study examines what might happen in an extreme situation, when perturbations such as warming could differentially affect the distinct components of the microbial food webs.

Characteristics of the *in situ* Arctic and Fjord waters

The chl *a* concentrations and abundances of bacteria, viruses, phototrophic and heterotrophic flagellates and ciliates in the Arctic and Fjord communities examined were comparable to values reported in other Arctic studies (Sherr et al. 1997, Middelboe et al. 2002, 2012, Lovejoy et al. 2007, Säwström et al. 2007b, Boras et al. 2010). BP (bacterial production) was significantly lower in open Arctic waters than in Fjord waters (Table 1), where the temperature was higher. The range of the BP corresponds to the values obtained for the summer period in areas close to the Svalbard Islands (Boras et al. 2010) and in Franklin Bay (Canadian Arctic, Garneau et al. 2008). VP_L (viral lytic production) was higher in the Fjord waters than in the open Arctic waters, while VP_{LG} (lysogenic viral production) was only found in the Fjord system, where it was higher than VP_L (Table 1). This has not been observed in previous studies (Säwström et al. 2007b, De Corte et al. 2011). Finally, protist grazing in the Arctic community was higher than bacterial mortality due to viruses, as shown in Boras et al. (2010), unlike in the Fjord community, where the impact of viruses on bacterial communities was higher than that of bacterivores, as shown by Wells & Deming (2006). These results suggest that both types of bacterial 'predators' could play an important role in controlling bacterial communities.

Changes in microbial biomasses and activities during the experiments

Phytoplankton and bacterioplankton

The effects of warming on phytoplankton biomass (chl *a* concentration) as well as on the pigmented ciliate *Myrionecta* sp. decreased with increasing temperature in all the Arctic microcosms (Figs. 3A,B & 4G,H). The observed trends of chl *a* concentration agree with Müren et al. (2005), who found the high-

est phytoplankton biomass at 5.0°C, which then decreased with higher temperatures and reached a minimum at 10.0°C. In the same experimental microcosms as ours, A. Coello-Camba (unpubl.) reported that the phytoplankton community composition varied from a high abundance of large cells, such as diatoms and dinoflagellates, at low temperatures to low abundances at higher temperatures. In addition, our findings of high abundances of PF ≤2 µm (*Micro-monas* sp.) at 5.0°C in the Arctic, and at 2.5, 5.0 and 7.0°C in the Fjord microcosm are in agreement with observations made by Lovejoy et al. (2007) in the Arctic Ocean, where there are blooms of this picoflagellate during spring and summer. In the present study, BA (bacterial abundance) and BP (bacterial production) increased when chl *a* concentration decreased, probably due to the increase in organic matter from excretion or cell lysis of primary producers that might stimulate bacterial growth, as proposed by Hoppe et al. (2008). Moreover, empirical relationships between temperature and bacterial growth in natural bacterioplankton assemblages have shown that temperature is closely related to BP (White et al. 1991, Wiebe et al. 1992, Shiah & Ducklow 1994). In particular, at low temperatures small increases cause significant changes in prokaryotic growth rates (Morán et al. 2006, Kirchman et al. 2009). Indeed, these observations are in accordance with our results, which show that BA and BP significantly increase with a few degrees of temperature (Table 3). This could be because psychrophilic bacteria, which are able to grow at lower temperatures (0, 15.0 and 20.0°C, minimum, optimal and maximum temperatures, respectively), are replaced by psychrotolerant bacteria, which grow better at higher temperatures (Morita 1975). Warmer seawater might result in a change in the bacteria community and this could be the reason why we found an increase in BA and BP above 5.5°C (Figs. 4A,B & 5A,B). These results are in agreement with Krause et al. (1993), who observed shifts from psychrophilic to psychrotolerant bacterial communities during the replacement of summer water in the Weddell Sea, as well as a decrease in nutrients and the chl *a* concentration.

Viral lytic and lysogenic production

Bacterial and viral abundances in aquatic systems are usually positively correlated, which indicates that they are closely linked, and presumably the environmental parameters that influence bacterial assemblages could also affect the viral community (Wom-

mack & Colwell 2000, Weinbauer 2004, Pradeep Ram & Sime-Ngando 2010, Danovaro et al. 2011). In the Arctic microcosms, VA (viral abundance) followed BA (bacterial abundance), while in the Fjord microcosms VA decreased above the temperature of 5.5°C. This decay could be due to different causes, such as adsorption into host walls or into particles, as well as ingestion by pico/nanoflagellates (Weinbauer 2004). Also, it would also have to take into account lysogeny. Lysogenic bacteria have prophages (phage nucleic acid) incorporated into their genomes. When the lysogenic host is stressed (e.g. by environmental shifts) the prophage is induced and the lytic cycle activated, producing new viral infective particles. High lysogeny values were found in Antarctic lakes during winter (Lisle & Priscu 2004) and there are a variety of reports for the Arctic that found significant lysogeny during summer (Boras et al. 2010), but in other cases it was not detected at all (Säwström et al. 2007b). Furthermore, at different polar sites, lysogeny showed seasonal variations, with high rates in winter and spring (Laybourn-Parry et al. 2007, Säwström et al. 2007a). We therefore expected to find low VP_L (viral lytic production) and high VP_{LG} (lysogenic viral production) at the low experimental temperatures when bacterial abundance was low (Fig. 4A,B). However, our results showed the opposite trend in both systems (Fig. 5C,D). In the Arctic microcosms, when BA increased with temperature (around 5.5°C) and presumably a shift in the bacterial community occurred, VP_L decreased significantly, showing similar values as VP_{LG} (Fig. 5C). In the Fjord microcosms, VP_L was also important at low experimental temperatures (1.0°C), while VP_{LG} constituted 65% of the total viral production at the *in situ* temperature (~6.0°C). A plausible explanation is that between 5.5 and 7.0°C, viruses were 'comfortably installed' inside the active hosts as prophages, but when the temperature increased (to 8.5 and 10.0°C), the new warming conditions acted as an environmental stress factor, and the lysogenic cycle reverted to the lytic cycle. Moreover, the stimulation of bacterial growth at higher temperatures could be related to an increase in the nutrient concentrations, and therefore in VP (Pradeep Ram & Sime-Ngando 2008, 2010). In summary, it seems that at higher temperatures than ~7.0°C the lysogenic cycle reverts to a lytic cycle.

Bacterial losses

In the Arctic microcosms, protistan grazing decreased progressively up to 5.5°C (Fig. 6E) but be-

tween 5.0 and 7.0°C there was a shift and the bacterial grazing rates increased, corresponding to high values of BA, BP and lysogeny (Figs. 4A & 5A,C). Fluctuations in bacterivory with temperature were not reflected in changes in the total HF abundances. Nevertheless, we observed differences in the dynamics of the different HF size classes (Fig. 4E). Above 5.5°C, HF $\leq 2 \mu\text{m}$ decreased, and around 7.0°C, HF $> 5 \mu\text{m}$ increased (Fig. 4E). In the Fjord microcosms, there was a gradual increase in bacterivory as the temperature increased; however, like in the Arctic microcosms, this did not correspond to an increase in the total HF at different temperatures. Although HF are considered to be the main bacterivore microorganisms (Sherr & Sherr 2002), they also ingest prey larger than bacteria to maintain their biomass and growth (Vaqué et al. 2008), and thus trophic cascades could occur (Vaqué et al. 2004). For instance, HF $> 5 \mu\text{m}$ could feed on bacteria, on HF $\leq 5 \mu\text{m}$, and on other small prey such as *Micromonas* sp., which were very abundant in our experiments as in natural Arctic waters (Lovejoy et al. 2007). In addition, phagotrophic ciliates and large flagellates (i.e. *Gyrodinium* sp.) could prey on pico/nanoflagellates (HF, PF), controlling their abundances and shaping the community (size and composition). In polar systems, bacterivory appears to be an important factor in controlling the bacterial abundance during most of the year (Anderson & Rivkin 2001, Boras et al. 2010). Furthermore, several authors have also found that grazing rates increase with temperature in the Antarctic (Vaqué et al. 2009) and in cold waters (Newfoundland, Choi & Peters 1992). Their results are in agreement with our bacterivory responses to warming in Arctic and Fjord waters. However, in Arctic waters, we found that the effect of temperature on viral lysis was not large enough for it to surpass bacterivory, while in the Fjord microcosms at the *in situ* temperature, viral-induced mortality was significantly higher than mortality due to protists (Fig. 6B,D).

We think that it is necessary to carry out more research focused on different sources of bacterial mortality, particularly due to viruses, in different polar areas, at different seasons, as *in situ* as well as microcosm warming experiments. The results could be used to test and understand the function of viruses in the microbial shunt in these cold marine systems in order to generate predictive models of the effects of future global warming. Indeed, viruses could be a key biotic component influencing the feedback of climate change in the oceans because they supply dissolved nutrients in the euphotic zone, contributing to the recycled primary production and/or to the in-

crease in CO₂ due to the respiration of heterotrophic microbes (Danovaro et al. 2011).

CONCLUSIONS

The results of this experimental study show that heterotrophic and phototrophic microbial communities responded differentially to warming conditions in 2 contrasting Arctic systems. After a gradual increase in temperature we observed a significant increase in the activities and biomasses of heterotrophic microorganisms, and a decrease in biomasses of phytoplankton. Under warmer conditions, bacteria were mainly channeled to higher trophic levels via HF, while viral lysis contributed to increasing the pool of dissolved organic matter in the water column. All the observed changes were larger in the open Arctic waters than in the Fjord waters, with different initial microbial communities and lower and higher temperatures, respectively. In conclusion, warming triggers shifts that would favor heterotrophic communities, which could have a large impact on carbon and nutrient cycling and carbon storage in the Arctic Ocean.

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