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Seasonal changes in planktonic bacterivory rates under the ice-covered coastal Arctic Ocean

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Abstract

Bacterivory was determined in surface waters of Franklin Bay, western Arctic, over a seasonal ice-covered period (winter–spring, 2003–2004). The objectives were to obtain information on the functioning of the microbial food web under the ice, during winter (from 21 December 2003 to 21 March 2004) and during spring (from 22 March 2004 to 29 May 2004), and to test whether bacterial losses would increase after the increase in bacterial production following the spring phytoplankton bloom. Chl *a* concentrations ranged from 0.04 to 0.36 μg L^{-1}, increasing in March and reaching a peak in April. Bacterial biomass showed no consistent trend for the whole period, and protist biomass followed a pattern similar to that of Chl *a*. Bacterial production increased 1 week after Chl *a* concentrations started to increase, while bacterivory rates increased very slightly. Average bacterivory rates in winter (0.16 $\pm$ 0.07 μg C L^{-1} d^{-1}) were not significantly different from those in spring (0.29 $\pm$ 0.24 μg C L^{-1} d^{-1}). Average bacterial production, on the other hand, was similar to bacterivory rates in winter (0.19 $\pm$ 0.38 μg C L^{-1} d^{-1}), but higher than bacterivory in spring (0.93 $\pm$ 0.28 μg C L^{-1} d^{-1}). Therefore, bacterial production was controlled by grazers during winter and by substrate concentration in spring.

The functioning of the microbial food web has been widely studied in diverse temperate to polar marine systems (Vaqué et al. 1994; Leakey et al. 1996; Strom 2000) and the key role of protists and viruses as the main causes of bacterial mortality has been recognized (Bird and Karl 1999; Pedrós-Alió et al. 2000; Wells and Deming 2006). Most studies on the importance of the microbial loop and the viral shunt in polar regions have been carried out in the summer period (Steward et al. 1996; Guixa-Boixereu et al. 2002). Winter data are scarce, but they indicate that the microbial community responds to this extreme seasonality by increasing both biomass and metabolic rates in spring compared to winter. For example, Kang et al. (1997), collected data over 366 d in Maxwell Bay (King George Island, Antarctica) describing the seasonal cycles of nearshore biomass of microbial assemblages as well as physicochemical variables. Sherr et al. (2003), and Sherr and Sherr (2003), as part of the year-long SHEBA/Jois drift experiment, analyzed abundance and biomass of autotrophic and heterotrophic microbes and bacterial activity in the upper 120 m of the water column of the ice-covered central Arctic Ocean. Bacterioplankton communities responded to the seasonal changes with increasing abundance and biomass (Sherr et al. 2003), along with a 4-fold increase in respiration and a 10-fold increase in production rates (Sherr and Sherr 2003). As part of the Canadian Arctic Exchange Study (CASES) Riedl and Gosselin (2007) reported bacterivory rates for the ice microbial communities and found higher specific ingestion rates at the beginning of the spring than in winter and late spring. Similarly, Wells and Deming (2006) studied bacterivory vs. viral lysis in bottom waters of Franklin Bay during winter, and suggest that viruses are the most important agents of bacterial mortality, at least during one period of winter. Even considering these limited studies there is essentially no

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Table 1. Surface values of Chl *a* concentration, abundance of bacteria (B), heterotrophic nanoflagellates (HNF), phototrophic nanoflagellates (PNF), ciliates, grazing rates (G), and bacterial production (BP) in Franklin Bay from Dec 03 to May 04. Average (±SD) triplicate subsamples. ND, not detectable; —, no data.

Date	Chl *a* (μg L^{-1})	B±SD (cells $L^{-1}\times10^8$)	HNF (cells $L^{-1}\times10^5$)	PNF (cells $L^{-1}\times10^5$)	Ciliates (cells L^{-1})	G±SD (cells $L^{-1}\times10^8$ d^{-1})	BP±SD (cells $L^{-1}\times10^8$ d^{-1})
22 Dec 03	0.10	2.52±0.21	3.86	0.82		0.29±0.00	0.01±0.00
11 Jan 04	0.04	1.74±0.04	3.30	1.53	333	0.29±0.03	2.29±0.29
17 Jan 04	0.04	2.39±0.79	2.11	0.56	370	0.37±0.24	−0.37±0.19
23 Jan 04	0.04	2,13±0.20	7.55	1.29	93	0.23±0.12	0.64±0.39
29 Jan 04	0.04	3.49±1.96	3.09	0.93	302	0.23±0.03	0.16±0.01
04 Feb 04	0.05	2.62±0.66	2.25	0.28	290	0.21±0.00	0.19±0.00
14 Feb 04	0.05	2.49±1.26	1.88	0.31	288	0.25±0.00	0.05±0.00
03 Mar 04	0.10	3.39±0.00	—	—	1275	0.01±0.00	0.01±0.00
21 Mar 04	0.15	3.21±0.00	2.26	0.88	350	0.41±0.30	1.23±0.84
11 Apr 04	0.36	3.57±0.94	3.87	1.64	978	0.73±0.14	1.36±0.59
17 Apr 04	0.32	3.28±0.71	4.02	3.24	225	0.25±0.00	1.34±0.00
23 Apr 04	0.28	4.53±1.36	6.45	4.54	175	0.38±0.36	1.43±1.04
29 Apr 04	0.28	4.31±0.93	2.21	1.99	269	0.78±0.00	0.92±0.12
05 May 04	0.26	2.03±0.00	4.51	1.91	317	ND	1.45±0.37
10 May 04	0.23	1.49±0.00	—	—	280	ND	0.41±0.14
22 May 04	0.20	3.61±0.00	—	—	688	0.25±0.00	1.28±0.20
27 May 04	0.30	5.16±0.00	3.23	3.11	1292	0.83±0.34	1.78±0.31

data on the question of how changes in the bacterioplankton community affect other components of the planktonic food web. Anderson and Rivkin (2001) summarized grazing mortality of bacterioplankton in high latitudes using limited published data along with their own studies (*see* table 1 in Anderson and Rivkin 2001) and found some temporal patterns common to both the Arctic and the Southern Ocean. They proposed the following sequence of events: (1) during late winter and early spring bacterial biomass (BB) and growth rates are low and grazing mortality is relatively high, so that protists consume most of the bacterial production ($\mu \leq$ g); (2) in spring, as solar radiation increases and picophytoplankton become more available, protist ingestion of picophytoplankton increases; this induces changes in the dissolved organic matter stoichiometry, and the grazing mortality on the nutrient-limited bacteria decreases ($\mu >$ g); (3) during the summer phytoplankton bloom, bacteria become nutrient-replete and grazing mortality again achieves the same order of magnitude as growth rate ($\mu \leq$ g); (4) after the bloom (late summer), as inorganic nutrients are being depleted, bacteria return to a nutrient-stressed state and, once more, both growth and grazing rates decrease with respect to the summer, with the situation similar to that in spring ($\mu >$ g). However, there are no data for winter bacterioplankton grazing mortality and, therefore, the picture remains incomplete.

The 2003–2004 CASES project was an opportunity to investigate the temporal variation in abundance, biomass, grazing rates, and production rates of planktonic microorganisms at a fixed station in Franklin Bay (Canadian Arctic). The station was ice-covered from winter (21 December 2003–21 March 2004, with a daily light period ≤12 h) through spring (22 March 2004 –29 May 2004, with a light period >12 h). The objectives of this study were: (1) to confirm the strong existence of seasonal changes in the microbial variables reported in earlier studies (Sherr and Sherr 2003; Sherr et al. 2003); (2) to test whether grazing losses of bacteria increased following the phytoplankton bloom and increased bacterial production; (3) to determine to what extent grazing rates on bacteria were important in regulating bacterial production during the ice-covered period; and (4) to estimate whether bacterial carbon consumption fulfilled protist carbon demands for growth. To accomplish these objectives, phytoplankton biomass, bacterial and protist abundance and biomass, bacterial production, and bacterivory rates were determined two to four times per month from the winter solstice to 29 May, when ice conditions allowed the ship to leave its overwintering site.

Methods

Sampling site—Samples were collected in Franklin Bay, Western Arctic (70°1.3′N, 126°25.2′W) 17 times between 22 December 2003 and 29 May 2004 (Table 1) on board the icebreaker CCGS *Amundsen*, which remained stationary following its deliberate freezing into land-fast ice. This permitted under-ice sampling of the water column every 6 d over nearly 6 months. Water from 3-m depth was collected with a Niskin bottle from a hole in the ice, 500 m away from the ship, in the upstream direction of the dominant current flow to minimize any influence from the ship. Maximal ice thickness was 1.7 m. Surface irradiance measurements were taken from an ice camp located 1 km from the ship (data provided by T. Papakyriakou, University of Manitoba, and S. Brugel, Université du Québec à Rimouski). In the 3-m samples water temperature was measured in situ with a mercury thermometer. Information of the rest of the water column was available from conductivity, temperature, and depth (CTD) casts through the moon pool in the ship.

Nutrient concentrations—Water for nutrient analysis was collected into single 15-mL acid-rinsed Falcon™ centrifuge tubes after prefiltration through a 0.20-μm Sartorius Minisart syringe filter. Nitrate + nitrite (nitrate), soluble reactive phosphorus (SRP), and silicate were analyzed onboard the ship, using a Brann and Luebbe 1 autoanalyzer II and standard protocols (Grasshoff 1999). For more details see Terrado et al. (in press).

Chlorophyll a concentration—250–500-mL water samples were filtered through 25-mm diameter Whatman GF/F filters and frozen at -20°C. The filter was thawed in 90% acetone for 24 h in the dark at 4°C, until chlorophyll *a* (Chl *a*) was extracted and determined fluorometrically (Parsons et al. 1984). No replicates were measured. However, values were compared to those of other measurements carried out independently and they were always very similar.

Abundance and biomass of microorganisms—Samples for bacteria, nanoflagellate, and ciliate abundance and biomass, as well as for bacterivory rate estimations, were collected two or four times every month. Subsamples of 100 mL were fixed with glutaraldehyde (1%, final concentration); triplicates of 20 mL were used for bacterioplankton and single aliquots of 60–80 mL for nanoflagellate counts. These were filtered through 0.2-μm and 0.6-μm black polycarbonate filters for bacteria and nanoflagellates respectively, and stained with 4,6-diamidino-2-phenylindole (DAPI; Porter and Feig 1980) at a final concentration of 5 μg mL^{-1} (Sieracki et al. 1985). Abundance of these microorganisms was determined at 1000× magnification either with an Olympus BX-60 (on board the CCGS *Amundsen*, and at Laval University, Quebec) or with an Olympus-BX40-102/E epifluorescence microscope (at the Institut de Ciències del Mar [CSIC] in Barcelona). Bacteria were counted using the UV excitation filter. BB was estimated using the equation of Norland (1993; *see* grazing rates section). Heterotrophic nanoflagellates (HNF) and phototrophic nanoflagellates (PNF) were counted under both UV excitation (blue fluorescence) and blue excitation (B2 filter). Under blue light we could discriminate PNF (showing red-orange autofluorescence, and/or plastidic structures) from colorless HNF. With this method we could not distinguish mixotrophic nanoflagellates. Random 10-mm transects (100-μm width) were examined and cells counted in each filter. Between 20 and 100 HNF and between 10 and 50 PNF per filter were counted, and were grouped into four size classes: $\leq$2 μm, 2–5 μm, 5–10 μm, and 10–20 μm. Cell volumes were estimated using the nearest geometrical figure, and the carbon content was estimated using a volume : carbon ratio of 0.22 pg C μm^{-3} (Børsheim and Bratbak 1987). Finally, 1 liter of sample was collected and kept in a 1.5-liter polyethylene bottle and immediately fixed with acidic lugol (2% final concentration). This was allowed to settle down for 48 h, and the supernatant was gently removed, leaving 200 mL. Half of this concentrate was settled in 100 mL chambers for at least 48 h before enumeration, at 400× magnification, using an inverted microscope (Zeiss). Ciliates were identified to genus level when possible: *Strombidium*, *Laboea* and *Tontonia* were grouped as oligotrichs (subclass Oligotrichia); *Strobilidium* and tintinnids were grouped as choreotrichs (subclass Choreotrichia); *Mesodinium* (subclass Haptorida) and scuticociliates (subclass: Scuticociliatia) were counted separately (Lynn and Small 2000). Ciliate average size was determined after measuring all cells recorded per sample. Ciliate volume was estimated by approximation of each cell to the nearest geometric shape. To avoid the probable underestimation of biovolume because of fixation with Lugol's solution (Leakey et al. 1994; Stoecker et al. 1994), the average cell volume for each identified group was converted to carbon equivalents using the factor experimentally derived for Lugol's fixed marine oligotrichs, 0.2 pg C μm^{-3} (Putt and Stoecker 1989). Carbon weight for tintinnids was estimated using the experimentally determined factor of 0.053 pg C μm^{-3} (Verity and Langdon 1984).

Grazing experimental setup—We collected 5 liters of seawater to estimate bacterivory rates in grazing experiments. Once on board, 1-liter subsamples were put into five 1.5-liter polyethylene bottles. Two bottles served as controls after the water was filtered through a 0.8-μm filter to remove grazers. All bottles (2 controls and triplicates) were inoculated with fluorescent-labeled bacteria (FLB) at 20% of the natural bacterial concentration. FLB were prepared with a culture of *Brevundimonas diminuta* (strain obtained from the Spanish Type Culture Collection, http://www.cect.org/index2.html, Burjassot, València). *B. diminuta* was heat-killed and stained with 5-([4,6 dichlorotriazin-2yl] amino)-fluorescein (DTAF; Vázquez-Domínguez et al. 1999). The bottles for the grazing experiment were incubated at -0.5°C and in the dark for 24 h. In situ temperature remained constant at ca. -1.7°C throughout the study. Bacterivory rates were determined following the disappearance of FLB as described in Vázquez-Domínguez et al. (1999). Briefly, from each triplicate incubation bottle and each duplicate control bottle we removed 1.8-mL aliquots at times 0 and 24 h, and immediately fixed these with 1% paraformaldehyde plus 0.05% glutaraldehyde (final concentration) for 10 min, followed by fast-freezing in liquid nitrogen with final storage at -80°C. Aliquots were divided into two fractions for counts of bacteria and FLB using a Becton Dickinson FACScalibur bench cytometer with a laser emitting at 488 nm. For DTAF-stained FLB counts, 200-μL subsamples were run at high flow rate (around 60 μL min^{-1}), with no added dye. To count heterotrophic bacteria, 200 μL was stained with a dimethyl sulfoxide–diluted Syto 13 (Molecular Probes) stock (10 : 1) at 2.5 μmol L^{-1} final concentration, left for at least 10 min in the dark to complete the staining, and run in the flow cytometer at low flow rate (around 24 μL min^{-1}). This provided in situ bacteria plus FLB. The number of natural bacteria was calculated by subtraction of the FLB counts. Data were acquired in log mode for 2 min for both bacteria and FLB. The abundance was determined using flow speed. The flow was calibrated every 10 samples by determining sample volume before and after a 10-min run. In all cases we added 10 μL of a solution of yellow-green 1-μm Polysciences latex beads (10^6

beads mL^{-1}) as an internal standard. Also, in parallel, some aliquots of 50 mL were taken, fixed with glutaraldehyde (1% final concentration), and observed by epifluorescence to check whether FLB were evenly distributed and not clumped. Bacterial cell size was estimated using the relationship between average bacterial size (obtained by image analysis of DAPI preparations following common procedures) and average green (FL1) fluorescence of the SYTO-13 stained sample relative to beads (Gasol and del Giorgio 2000):

$$\mu m^3\ cell^{-1} = 0.0075 + 0.11 \times (\text{FL1 bacteria}/\text{FL1 beads}) \quad (1)$$

BB was calculated by using the carbon to volume relationship derived by Norland (1993) from the data of Simon and Azam (1989):

$$pg\ C\ cell^{-1} = 0.12\ pg \times (\mu m^3\ cell^{-1})^{0.7} \quad (2)$$

Bacterial carbon content varied between 7.0 and 9.0 fg C $cell^{-1}$. The cell size for the time zero obtained in each grazing experiment was used to estimate the in situ BB. *B. diminuta* are small rod-shaped cells of around 1.07-μm length and 0.29-μm width, with a volume of ca 0.065 μm^3 $cell^{-1}$ (Vázquez-Domínguez et al. 1999).

Grazing rates and bacterial production—The amount of bacteria grazed was obtained following the mathematical model #3 of Salat and Marrasé (1994), first computing:

$$g = -(1 / t) \times \ln(FLB_t / FLB_o) \quad (3)$$

where g is specific grazing rate (d^{-1}), t is incubation time, FLB_t is number of FLB at the considered incubation time, and FLB_o is number of FLB at the initial time.

$$a = (1 / t) \times \ln(B_t / B_o) \quad (4)$$

where a is specific bacterial net growth rate (d^{-1}) of the whole sample, t is incubation time, B_t is bacterial number at the final time, and B_o is bacterial number at the initial time considered.

$$k = a + g \quad (5)$$

where k is specific growth rate of bacteria (d^{-1}), assuming that bacterivory was the main loss factor for bacteria. And then,

$$G = (g / a) \times \Delta B_t \quad (6)$$

where G is total grazing (bacteria consumed $L^{-1}\ d^{-1}$) and ΔB_t is the net bacterial production per day.

$$\Delta B_t = B_0 \times (e^{at_1} - 1) \quad (7)$$

where $t_1 = 1$ d.

$$BP = \Delta Bi + G \quad (8)$$

where BP is bacterial production in the incubation bottles (bacteria produced $L^{-1}\ d^{-1}$).

For each interval time, grazing and production rates of bacteria were unified as bacteria consumed or produced $L^{-1}\ d^{-1}$ or converted to biomass (μg C $L^{-1}\ d^{-1}$).

Finally, specific ingestion rates (bacteria $HNF^{-1}\ h^{-1}$), assuming that grazing on bacteria was mainly because of HNF, were calculated from total grazing rates (G, bacteria $L^{-1}\ d^{-1}$) divided by HNF L^{-1} (assuming that HNF were the only bacterivores) and by 24 h to convert daily consumption to hourly cell ingestion.

$$\text{Specific ingestion rate (B HNF}^{-1}\ \text{h}^{-1}) = \text{G, B L}^{-1}\ \text{d}^{-1}\ /(\text{HNF L}^{-1} \times 24\ \text{h}) \quad (9)$$

Data analysis—The relationships between biological variables were examined by means of regression and correlation analyses computing Pearson pairwise statistics. Differences in biological variables (Chl *a* concentration, microbial biomass, grazing, and bacterial production rates) between winter and spring were tested with ANOVA.

Results

Background of the study area—Throughout the period of sampling from late December to the end of May, the water column was covered by ice. Water temperatures at 3 m ranged between -1.5°C and -1.8°C, and the salinity range was 30–31.5. Changes in temperature and salinity from surface to 220 m are described in Forest et al. (2006) and Garneau et al. (2008). Maximal ice thickness was 1.7 m in late March. A less saline upper layer ~15 m thick was present throughout Franklin Bay, and Chl *a* concentrations and bacterial abundance at 3 m, sampled through separate holes in the ice, were similar within an 18-km radius of the ship. Usually the under-ice reduced-salinity layer had higher bacterial activity and Chl *a* concentrations than deeper waters. Under ice, surface water irradiance increased over the sampling period from undetectable to 58 mol photons $m^{-2}\ d^{-1}$ (Garneau et al. unpubl.). Inorganic nutrient concentrations in the surface waters remained constant throughout winter and spring. Thus, nitrate concentrations were <3 μmol L^{-1} and SRP concentrations <1 μmol L^{-1}, and maximal values of silicate concentration were found in winter (ca. 10 μmol L^{-1}) with lower values in spring (7 μmol L^{-1}) (data provided by J.-E. Tremblay, N. M. Price, and K. Simpson, McGill University).

Abundance and biomass of microorganisms—Microbial abundance, biomass, and activities for the whole period are shown in Tables 1 and 2. Chl *a* concentrations during the ice-covered period ranged from 0.04 to 0.36 μg L^{-1}. The maximal value was in April, when the irradiance period was longer than 12 h d^{-1}, and the minimal value in January corresponded to the darkest period. There was a 10-fold increase in Chl *a* between mid-January and mid-April, followed by a small decrease from 0.36 to 0.20 μg L^{-1} during April and late May (Table 1; Fig. 1A). Thus, the average value of Chl *a* in winter was significantly lower than in spring (Table 2). Abundance and biomass of PNF

Table 2. Average ± SD of the mean for biomasses and activity rates in winter ($n = 8$) and in spring ($n = 9$).

Variable	Winter	Spring
Chl *a* (μg L^{-1})	0.06±0.02	0.25±0.07***
PNF biomass (μg C L^{-1})	0.94±0.19	5.94±2,81***
Bacterial biomass (μg C L^{-1})	1.81±0.50	2.56±0.81*
HNF (μg C L^{-1})	2.40±0.59	4.45±1.39**
Ciliate biomass (μg C L^{-1})	0.32±0.13	0.42±0.48
Bacterial production rates (bottles) (μg C L^{-1} d^{-1})	0.19±0.38	0.93±0.28***
Grazing rates (μg C L^{-1} d^{-1})	0.16±0.07	0.29±0.24

PNF, phototrophic nanoflagellates; HNF, heterotrophic nanoflagellates. * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$ (significant differences between winter and spring).

followed a similar trend to that of Chl *a* (Fig. 1A; Table 1). Pearson correlation analysis showed a positive and significant correlation between Chl *a* concentration and PNF biomass ($r = 0.832$, $p < 0.01$).

Bacterial abundance presented a 3-fold increase between minimal winter value in January and maximal spring value in May (Table 1). There was a drop in bacterial abundance during the first 2 weeks of May compared to the end of April but values recovered by the end of May. Average bacterial abundance in winter ($2.59 \times 10^8 \pm 0.59 \times 10^8$ cells L^{-1}) was slightly lower than in spring ($3.47 \times 10^8 \pm 1.10 \times 10^8$ cells L^{-1}). There were greater differences in average BB between winter and spring than in abundance (Table 2), attributed to the slightly lower cell biomass in winter compared to spring (data not shown). Maximal BB was detected in May (3.54 ± 0.58 μg C L^{-1}) and minimal in January (1.24 ± 0.04 μg C L^{-1}) (Fig. 1B). Although bacterial dynamics over the season were quite variable, BB was significantly correlated with Chl *a* concentration ($r = 0.539$, $p < 0.05$).

The abundance of HNF decreased 4-fold between January (7.55×10^5 cells L^{-1}) and February (1.88×10^5 cells L^{-1}), followed by an increase up to late April (6.45×10^5 cells L^{-1}) (Table 1). Average HNF biomass increased significantly between winter and spring (Table 2), showing the highest value in April and the minimum in February (Fig. 1B). HNF were more abundant and with greater biomass compared to PNF during the winter (from December to late March), representing 56–88% of total nanoflagellate biomass and 68–89% in terms of abundance. These percentages of HNF decreased when the irradiance period increased (from mid-March to late May). In this period HNF accounted for 37–50% of total nanoflagellate biomass, and 51–72% of abundance (Table 1; Fig. 1A,B). HNF biomass was greater than BB, as HNF cells larger than 5 μm (Fig. 1C) contributed to significant biomass. Smaller cells were numerically more common; between 76% and 96% of HNF were in size classes ≤2 to 5 μm. The HNF size class of 5–10 μm represented 5–20% of HNF abundance, and finally the size class >10 μm represented between 2% and 7% of total abundance. However, in terms of carbon, HNF with sizes >5 μm were the most important (Fig. 1C). HNF total biomass followed a similar trend to that of phytoplankton biomass, with the larger fractions

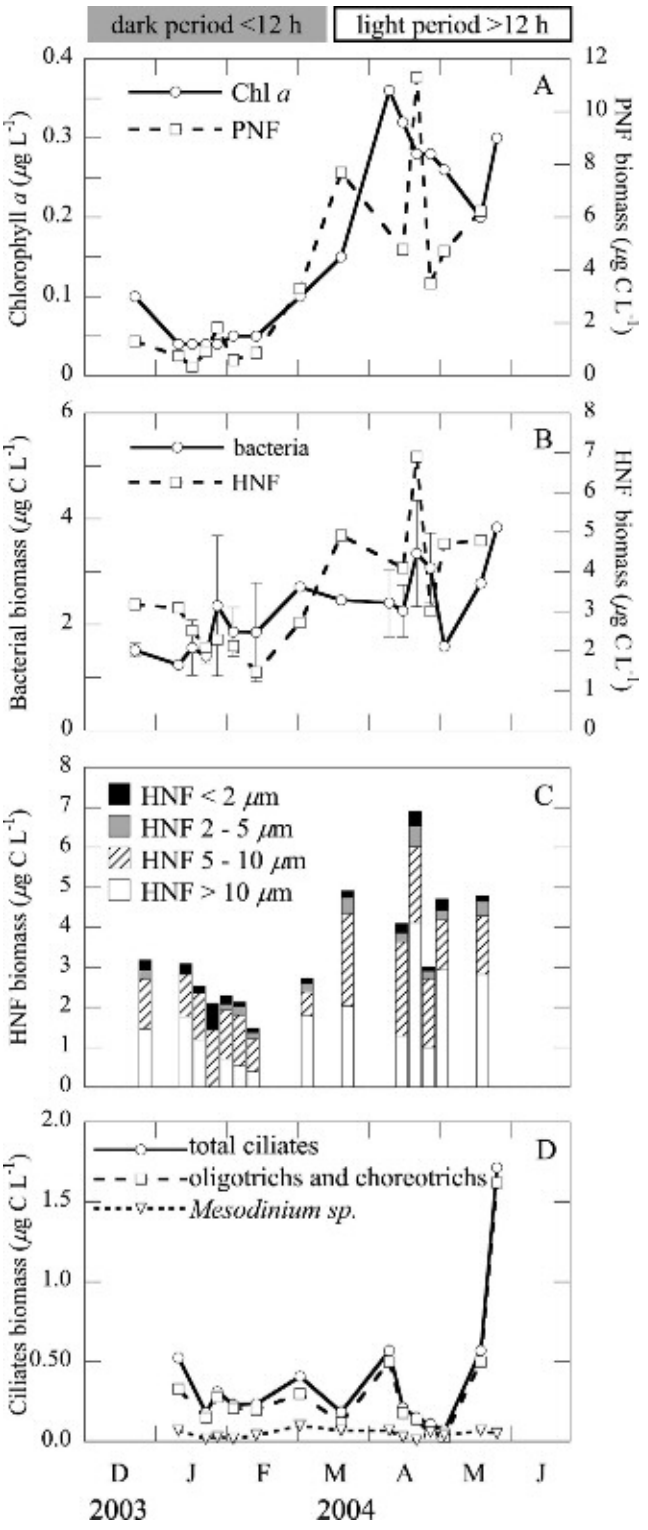

Fig. 1. Seasonal dynamics of microbial variables: (A) Chl *a* concentration and phototrophic nanoflagellate biomass (PNF); (B) mean bacterial biomass ± SD (triplicate ± standard deviation) and heterotrophic nanoflagellate biomass (HNF); (C) biomass corresponding to different HNF size classes; and (D) ciliate biomass (total, oligotrichs plus choreotrichs and *Mesodinium*). Length of the light period is shown in the upper part of the graph.

(HNF 5–10 μm and HNF >10 μm; Fig. 1C) contributing more to the totals. Thus, correlation coefficients between Chl *a* and HNF 5–10 μm ($r = 0.644$, $p = 0.01$) and HNF 10–20 μm ($r = 0.591$, $p < 0.05$) were both statistically significant and greater than those between Chl *a* and HNF 2–5 μm ($r = 0.469$, $p = 0.05$). BB and HNF biomass were significantly correlated with HNF 2–5 μm ($r = 0.566$, $p < 0.05$) but not with HNF >5–20 μm ($r = 0.419$, $p > 0.05$).

Ciliate abundance increased 14-fold from late January (93 cells L^{-1}) to the beginning of March (1275 cells L^{-1}), followed by a decrease through mid-April (175 cells L^{-1}), and an increase thereafter until the end of May (1292 cells L^{-1}). Both the maximal and the minimal biomass occurred in May (Fig. 1D). There were no significant differences between average values of total ciliate biomass in winter and spring (Table 2). The most common groups of ciliates were oligotrichs (*Strombidium*, *Laboea*, and *Tontonia*) and choreotrichs (*Strobilidium* and tintinnids), which together represented between 40% and 95% of total ciliate biomass (Fig. 1D). *Mesodinium* was significant in terms of abundance (from 10% to 88% of total ciliate abundance), but in terms of biomass it was less important than the oligotrichs and the choreotrichs (3–60%) (Fig. 1D). Occasionally we detected scuticociliates (data not shown), which accounted for up to 6% in terms of abundance and up to 25% of the total ciliate biomass when present.

Grazing and bacterial production rates—Disappearance of FLB in live controls (0.8 μm filtered water) was negligible in all experiments (data not shown). Changes in the specific net growth rates (a, d^{-1}) and specific grazing rates (g, d^{-1}) followed opposite trends (Fig. 2A) ($r = -0.529$, $p < 0.05$). In winter, almost all values of net growth rates were negative or close to zero, whereas specific grazing rates were positive (Fig. 2A). Specific net growth rates became higher than grazing rates as the intensity and duration of irradiance increased after spring started (Fig. 2A).

Bacterial production (BP) rates as cells produced L^{-1} d^{-1} reached both highest ($2.29 \pm 0.29 \times 10^8$ cells L^{-1} d^{-1}) and lowest values ($-0.37 \pm 0.19 \times 10^8$ cells L^{-1} d^{-1}) in January. This negative value for BP was the result of the sum of grazing rates (G) + net production (ΔBi), where ΔBi was a negative value higher than G (Eq. 8). This result suggests that there were alternative bacterial losses such as viral lysis. No significant changes in bacterivory rates (G) were detected between winter and spring, whereas bacterial carbon production rates were higher in spring than in winter (Table 2). Bacterial carbon consumed varied from undetectable at the beginning of May to 0.56 μg C L^{-1} d^{-1} at the end of May (Fig. 2B), and bacterial carbon production rates were at their minimum values in January (-0.24 ± 0.13 μg C L^{-1} d^{-1}) and a maximum value at the end of May (1.33 ± 0.01 μg C L^{-1} d^{-1}; Fig. 2B). BP increased 1 week after Chl *a* concentration started to increase (Figs. 1A, 2B; Table 1). However, this increase was not translated into an increase in bacterial grazing rates (Fig. 2B). Comparing specific growth rates (k = a + g) with specific grazing rates (g), there was a clear segregation of these variables between winter (≤12 h daily light) and spring (>12 h light). Specific grazing rates varied little over winter and spring, whereas specific growth rates were enhanced in spring (Fig. 3).

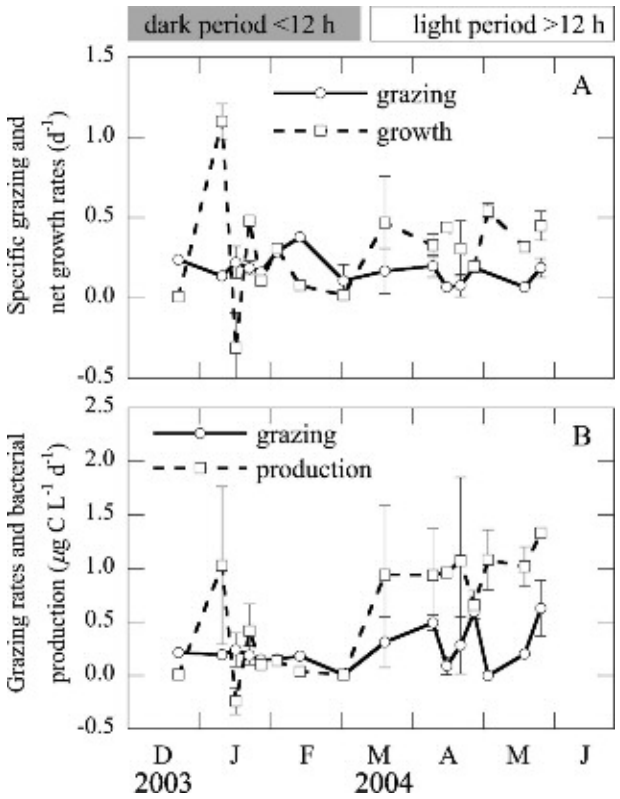

Fig. 2. Average values of activity rates ± SD (triplicate ± SD): (A) specific net growth and grazing rates; (B) bacterial production and grazing rates. Length of the light period is indicated.

Discussion

Methodological considerations—BB: Bacterial size was estimated following the equation given by Gasol and del Giorgio (2000), and BB was calculated by using the carbon to volume relationship derived by Norland (1993) from the data of Simon and Azam (1989). All these measurements and transformations are subject to potential errors. Such errors could affect the comparison of BP and grazing rates. We also calculated BP and grazing rates in terms of cells without converting to biomass. The results (Table 1) showed the same pattern as in Fig. 2B. Thus, calculation of biomass did not affect the comparison of these two variables. In addition, the values of carbon content per cell varied between 7.0 and 9.0 fg C $cell^{-1}$, and were similar to those found in oligotrophic systems and in the low range for other aquatic systems, for example, in the oligotrophic Central Atlantic values were 10 fg C $cell^{-1}$ (Vázquez-Domínguez et al. 2005). Overall our results fit within the range of values from the Hawaii Ocean time series HOT site of 3.5–8.8 fg C $cell^{-1}$ (Christian and Karl 1994). However, higher values have been estimated in the central Arctic Ocean (between 19 and 43 fg C $cell^{-1}$; Sherr et al. 1997) and in Disko Bay (36 fg C $cell^{-1}$; Nielsen and Hansen

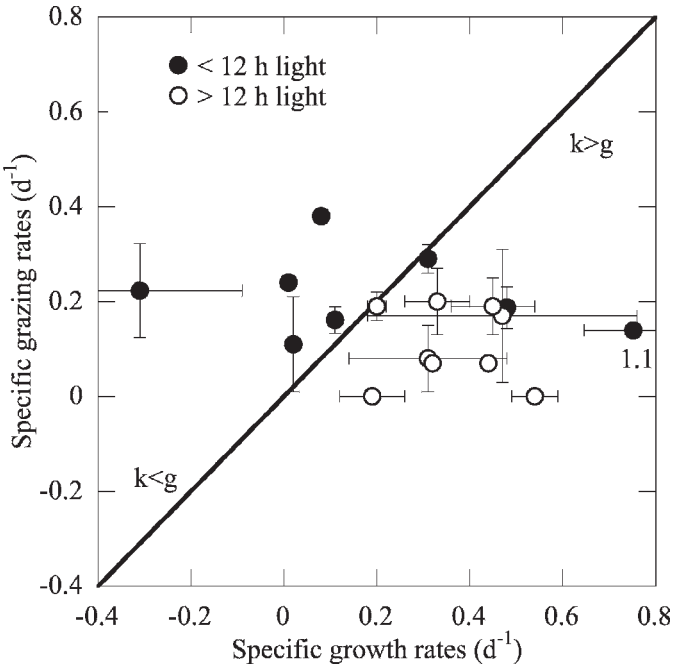

Fig. 3. Relationship between specific grazing rates (g) and specific growth rate (k). Closed symbols correspond to values between December and the beginning of March (≤12 h light). Open symbols correspond to values between mid-March and May (>12 h light). Error bars correspond to SD.

1995). The latter results were all from the summer season, after the ice melt, whereas our determinations were done over the winter–spring season in the surface water under the ice.

Grazing rates: The FLB disappearance technique (Pace et al. 1990) was developed to measure grazing rates on bacteria by the whole community of protists. The use of FLB as bacterial surrogates has known limitations, such as disturbance of the sample and underestimation of bacterial losses because of prey selection (Monger and Landry 1992; Christofferson et al. 1997). Despite this, the technique has been widely applied, and methodological limitations also apply to other techniques (Landry 1994; Vaqué et al. 1994, 2002*a*). In conclusion, we found that grazing rates were comparatively more important during winter than in spring. The alternative ingestion technique (direct counts of FLB inside the digestive vacuoles) would provide taxonomic information about actual grazers in addition to rates (Strom, 2000), but in our very oligotrophic system, this technique would most likely not have been sensitive enough (Marrasé unpubl.). We also compared the specific grazing and growth rates (Table 3) to other studies (Moisan et al. 1991; Bird and Karl 1999), and our results fell within the range of values.

Bacterial production: BP was estimated from the incubation bottles where grazing rates were determined and calculated as the sum of net production plus total grazing rates over 24 h of incubation (model 3, Salat and Marrasé 1994). Whereas estimates of bacterial production by ^{3}H-leucine are measured over short periods (~4 h), grazing determinations required much longer incubations to be measurable in these waters. For this reason we compared the consumed and actual bacterial increases from the same incubation bottles, rather than comparing the two activities (grazing and production) measured in incubations of different length. The limitation of the FLB method to estimate BP, discussed in Vaqué et al. (2002*a*,*b*), is that BP is likely underestimated because calculations are based on the conservative assumption that all bacterial losses are due exclusively to grazing by protists, and virus-induced mortality is not considered. Guixa-Boixereu et al. (2002) found that viral mortality was significant in Antarctic waters, and this may be true of polar waters in general. Wells and Deming (2006) analyzed viral mortality and bacterivory during March in near-bottom waters of Franklin Bay and reported that bacterial mortality caused by viruses could be at least twice as that caused by grazers. Including bacterial losses caused by viruses and grazers would imply greater total bacterial production, but we do not believe that this would change the overall pattern of BP over the winter-spring season.

Changes in abundance and biomass of planktonic microorganisms—Our winter and spring average biomass values in terms of Chl *a*, bacterial, and protist abundance were similar to other Arctic winter values (Sherr et al. 2003) but lower values than Southern Ocean summer values (Putt et al. 1991; Anderson and Rivkin 2001). Except for bacterial abundance and biomass, stocks of microorganisms from 3 m showed a strong seasonality similar to the central Arctic Ocean (Sherr et al. 2003) and Resolute Passage (Anderson and Rivkin 2001). Phototrophic and heterotrophic microorganisms increased significantly in early spring under the ice (Tables 1, 2; Fig. 1), consistent with the initial winter-spring bloom of phytoplankton triggering bacterial production, and increases in HNF and ciliate biomass.

Bacterivory and bacterial production rates. Bottom-up and top-down control—The control exerted by predators on bacterial production was clearly different in winter and in spring (Figs. 2, 3). Except for two occasions, specific growth rates during winter were equal to or lower than specific grazing rates ($k \leq g$), and consequently, the average percentage of bacterial carbon production consumed was high (152% ± 159% d^{-1}). In spring specific growth rates were equal to or higher than specific grazing rates ($k \geq g$), and the average percentage of bacterial carbon production consumed was low (31% ± 26%). These results suggest that bacterial production was controlled by grazing in winter and by carbon supply in spring. Anderson and Rivkin (2001) proposed a similar shift from late winter to spring. Here we show that their late winter scenario can be generalized to most of the winter in Franklin Bay. Billen et al. (1990) proposed that the relationship between BB and bacterial production could be used to examine the relative importance of resource supply (bottom-up control) vs. predation (top-down control) in determining the biomass of bacteria in any given system. This approach was expanded by Ducklow (1992) using data from very different areas of

Table 3. Ranges of bacteria, heterotrophic nanoflagellate (HNF) abundance, specific bacteria ingestion rates, specific bacterial growth (k) and grazing (g) rates, and temperature values recorded in different polar systems. Season and method used are included.

Location	Bacterial abundance ($\times 10^8$ L^{-1})	HNF abundance ($\times 10^6$ L^{-1})	Ingestion (bacteria HNF^{-1} h^{-1})	k (d^{-1})	g (d^{-1})	Temperature (°C)	Grazing method	Source
Ice edge								Moisan et al. (1991); Putt et al. (1991)
McMurdo Sound	1.0 to 7.0	1.0 to 12.0	0.01 to 2.4	−0.1 to 0.3	0.2 to 0.5	−1.0 to 0.4 (spring–summer)	BEADS (uptake)	
Atlantic Sector								Becquevort (1997)
Southern Ocean	4.3 to 6.4	0.3 to 1.4* 0.06 to 0.2†	0.9 to 1.3* 4.2 to 10.3†	— —	0.1* 0.2†	no data (spring)	FLB (uptake)	
East Antarctica								Leakey et al. (1996);
Prydz Bay	2.1 to 8.3	1.6 to 4.2	0.06 to 8.3	—	—	−1.4 to (−0.4) (summer)	FLB (uptake)	
Antarctic Peninsula								Vaqué et al. (2002*a*)
Ice Edge	0.7 to 10.0	0.0 to 1.3	nd to 66.9	0.1 to 0.5	nd to 0.4	−1.7 to −0.1	MINIS	
Weddell Sea	0.7 to 11.5	0.3 to 5.6	nd to 9.6	−0.4 to 1.2	nd to 0.8	−0.5 to 0.7	MINIS	
Front	1.5 to 7.6	0.8 to 9.7	0.5 to 2.2	0.1 to 0.4	0.05 to 0.1	−1.1 to 1.7	MINIS	
BSO to WSC	1.4 to 6.2	0.2 to 11.8	1.1 to 7.5	0.2 to 0.5	0.05 to 0.2	0.04 to 2.4 (summer)	MINIS (disappearance)	
Antarctic Peninsula								Vaqué et al. (2002*b*)
Bellingshausen	3.4 to 3.5	0.3 to 2.2	1.8 to 6.4	0.1 to 0.6	0.03 to 0.2	−0.06 to 2.4	MINIS	
Bransfield Strait	3.6 to 6.9	0.5 to 0.9	0.1 to 7.8	0.08 to 0.1	0.01 to 0.1	0.0 to 0.2	MINIS	
Gerlache Strait	2.5 to 7.2	0.4 to 2.1	4.7 to 13.2	0.3 to 0.4	0.09 to 0.1	−1.2 to 0.4 (spring–summer)	MINIS (disappearance)	
Antarctic Peninsula								Bird and Karl (1999)
Gerlache Strait	0.9 to 7.5	0.5 to 2.6	1.4 to 4.4	−0.6 to 0.3‡	0.05 to 0.2	−0.5 to 0.8 (spring)	FLB (uptake)	
Arctic Ocean	3.8 to 11.7	0.2 to 1.9	0.16 to 1.0	—	—	no data (summer)	FLB (uptake)	Sherr et al. (1997)
Canadian Arctic								Laurion et al. (1995)
Resolute Passage (Sea ice)	1.0 to 10.0	0.2 to 35.0	≤ 3 to 64	0.1	—	no data (spring)	FLB (disappearance)	
Canadian Arctic								This study
Franklin Bay	1.5 to 5.2	0.2 to 0.6	1.3 to 14.8	−0.5 to 0.5	nd to 0.4	−1.7 to −1.5 (winter–spring)	FLB (disappearance)	

nd, not detectable; —, no data. Fluorescent particles used: BEADS, fluorescent plastic beads; FLB, fluorescent-labeled bacteria; MINIS, fluorescent minicells.
*HNF < 5 μm.
†HNF >5 μm.
‡Net growth rate.

the world's ocean. He concluded that for log transformed data of BB (in μg C L^{-1}) plotted against bacterial production (BP in μg C L^{-1} h^{-1}), regression slopes less than 0.4 indicate weak control by resource supply, slopes between 0.4 and 0.6 indicate a moderate degree of resource limitation, and slopes greater than 0.6 indicate strong control by resource supply. Following this convention we compared the winter and spring data by regression analysis between our BB and BP data. In winter the relationship between BB and BP rates was not significant, consistent with top-down control. In spring, the slope of the regression line was 0.6, suggesting a significant control by resource supply (Fig. 4). This conclusion, however, should be taken with caution because of the few data points. Garneau et al. (2008), who estimated BP using ^{3}H-leucine incorporation, also compared the slopes of the regression lines between BB and BP throughout the water column and concluded that bacteria were resource limited (bottom-up control) in summer-autumn, but not in winter-spring. The discrepancy between the different relationships encountered for our and their data sets of BB and BP is because of the different length of time considered. If we had pooled data for spring plus winter we would have also found no significant relationship for the winter-spring period and, thus, an indication of top-down control. But separation into two periods uncovered the significant relationship for spring. The increase in bacterial production in the ice-covered surface water over the winter-spring period tracked the duration and intensity of irradiance as the season progressed. During this period of increasing light, nutrients might also be released from the ice and spur a phytoplankton bloom. Indeed, this surface water was highly influenced by the ice cover; there was little or no vertical mixing of the upper mixed layer low-salinity polar waters over the study period (Forest et al. 2006), and both temperature and salinity remained low at ca. -1.7°C and 31, respectively.

Effect of heterotrophic nanoflagellates on bacteria—Anderson and Rivkin (2001) proposed that grazing mortality for bacteria changes with season in polar waters. Using a dilution technique to estimate grazing rates, they hypothesized that before and after the initial irradiance-induced phytoplankton bloom the bacterioplankton were nitrogen limited and protists avoided N-stressed bacteria and grazed on algae. Thus, protist grazers would alternate between the bacterivorous and herbivorous nutritional modes as relative nutritional value of the potential prey changed. Using our FLB technique, we were not able to estimate algal vs. bacterial prey preference by the grazer community. We note, however, that HNF and ciliates increased in biomass just after the Chl *a* concentration peak in early spring, in parallel with a very slight increase in bacterivory rates (Figs. 1A,B,C, 2B). We also note a significant correlation between Chl *a* concentration and biomass of HNF >5 μm, and the covariation could be because of HNF grazing on photosynthetic microorganisms in addition to bacteria. The small prasinophyte *Micromonas* sp. (1.5–3 μm) persisted throughout winter darkness and maintained steady exponential growth from late winter to early summer in Franklin Bay (Lovejoy et al. 2007) and was likely part of the HNF ≥ 5 μm diet (Sherr et al. 1997). There was also a significant correlation between BB and biomass of HNF <5 μm, suggesting that the smallest HNF were the most important bacterivores, as reported elsewhere (Sherr and Sherr 2002). In order to look at the effect of different size classes of HNF on bacteria, we calculated the specific ingested cells HNF^{-1}. We assumed that ciliates were not important grazers of bacteria because only a small proportion of the ciliates counted such as small scuticociliates are considered strictly bacterivorous and these were not always present. Without ciliates, grazing on bacteria would be mainly by HNF. Then we carried out calculations in two different ways, (1) bacteria were ingested only by HNF ≤ 5 μm, or (2) bacteria were ingested by the whole HNF assemblage. Each HNF ≤ 5 μm consumed on average 4.7 $\pm$ 2.3 bacteria h^{-1} (1.4–9.1 bacteria HNF^{-1} h^{-1}) in winter and 9.5 $\pm$ 6.0 bacteria h^{-1} (2.8–19.3 bacteria HNF^{-1} h^{-1}) in spring. When considering the whole community of HNF, the number of ingested cells was 4.0 $\pm$ 1.8 bacteria HNF^{-1} h^{-1} (1.3–5.5 bacteria HNF^{-1} h^{-1}) in winter and 7.6 $\pm$ 4.5 bacteria HNF^{-1} h^{-1} (2.6–14.8 bacteria HNF^{-1} h^{-1}) in spring. These values are similar to and slightly higher than other estimates for polar waters (Table 3). For example, in Prydz Bay, HNF consumed 0.08–8.3 bacteria h^{-1} (Leakey et al. 1996). In the Atlantic sector of the Southern Ocean, Becquevort (1997) estimated an average of 1.1 bacteria h^{-1} for HNF <5 μm and 7.3 bacteria h^{-1} for HNF >5 μm, and Bird and Karl (1999), obtained 1.4–4.4 bacteria HNF^{-1} h^{-1} including colonial flagellates in the Gerlache Strait. Ingestion rates estimated for the Arctic Ocean ranged from 0.16 to 1 bacteria HNF^{-1} h^{-1} (Sherr et al. 1997). Higher values were reported from sea ice in Resolute Passage (Canadian Arctic), with ingestion rates from ≤ 3 to 64 bacteria HNF^{-1} h^{-1} (Laurion et al. 1995), and in Franklin Bay (Riedl and Gosselin 2007). Except for the sea ice in the Arctic and the ice edge in Antarctica, all these values (Table 3) were measured using the FLB disappearance method or the direct uptake of fluorescent-labeled particles method. Regardless of the method used, the values are similar to or lower than values from oligotrophic systems in lower latitudes ($\sim$8 bacteria HNF^{-1} h^{-1}, Vaqué et al. 1994).

To test the importance of bacteria in the HNF diet, we estimated whether the observed consumption of bacteria was sufficient to satisfy the carbon requirements for the net growth of the HNF population. We used the following equation:

$$\text{Carbon demand } (\mu\text{g C L}^{-1}\text{ d}^{-1}) = \mu \text{ Bv C} / 0.40 \quad (10)$$

where 0.40 is the assumed growth efficiency (Sanders et al. 1992); μ (d^{-1}) is the HNF net growth rate; Bv (μm^3 L^{-1}) is the HNF assemblage biovolume; and C is the carbon content of HNF μm^{-3} (Børsheim and Bratbak 1987). The HNF net growth rate used was 0.18 d^{-1}, obtained by Laurion et al. (1995), close to the values obtained in Antarctic waters (0.2 d^{-1}) by Vaqué et al. (2002*b*).

When grazing was detectable we found that bacterial carbon ingestion almost always could satisfy the carbon

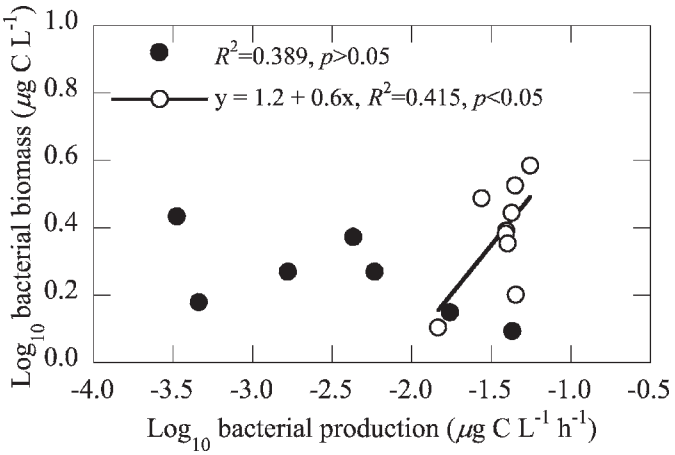

Fig. 4. Relationships between bacterial biomass (BB) and bacterial production (BP). Closed symbols correspond to values between December and the beginning of March (≤12 h light). Open symbols correspond to values between mid-March and May (>12 h light).

demand of HNF ≤5 μm both in winter (100% ± 34%), and in spring (117% ± 142%, Fig. 5). However, HNF >5 μm always needed an extra input of carbon. The ingested bacterial carbon for this size fraction only represented an average of 19% ± 6% of their carbon demand in winter and 14% ± 15% in spring.

HNF carbon demand was estimated under conditions that, in principle, should favor a good balance between bacterial carbon consumption and HNF carbon requirement. On the one hand, HNF carbon requirements were probably underestimated, because they were based on HNF net growth rate without taking into account HNF mortality, and on the other hand, we assumed that HNF were the only bacterivores without taking into account that a small fraction of the bacteria could have been grazed by ciliates. These two considerations would favor a relatively high bacterial carbon to HNF requirement. Thus, our conclusion is robust.

This gives further support to the idea that the HNF >5 μm were mainly algivores and not bacterivores. It also indicates that there were two trophic chains: one from bacteria to HNF ≤5 μm, and another from larger prey than bacteria such as small algae to HNF >5 μm. This intriguing notion should be tested using more targeted grazing experiments. Another factor that could complicate the microbial food webs would be grazing by larger HNF on the smaller size fraction of HNF. We found that a large proportion of the HNF were very small, and Lovejoy et al (2006) reported a diverse community of heterotrophic picoeukaryotes (>3 μm) in Arctic waters. The edibility and nutritional importance of these small cells for larger HNF is unknown.

Despite low Chl *a* concentration in the ice-covered water, the microbial community persisted throughout winter in the Arctic environment. The winter community was dominated by heterotrophic microorganisms, but also contained a small community of phototrophs. We detected a strong seasonality in phototroph and HNF biomass and bacterial production during the winter–spring transition.

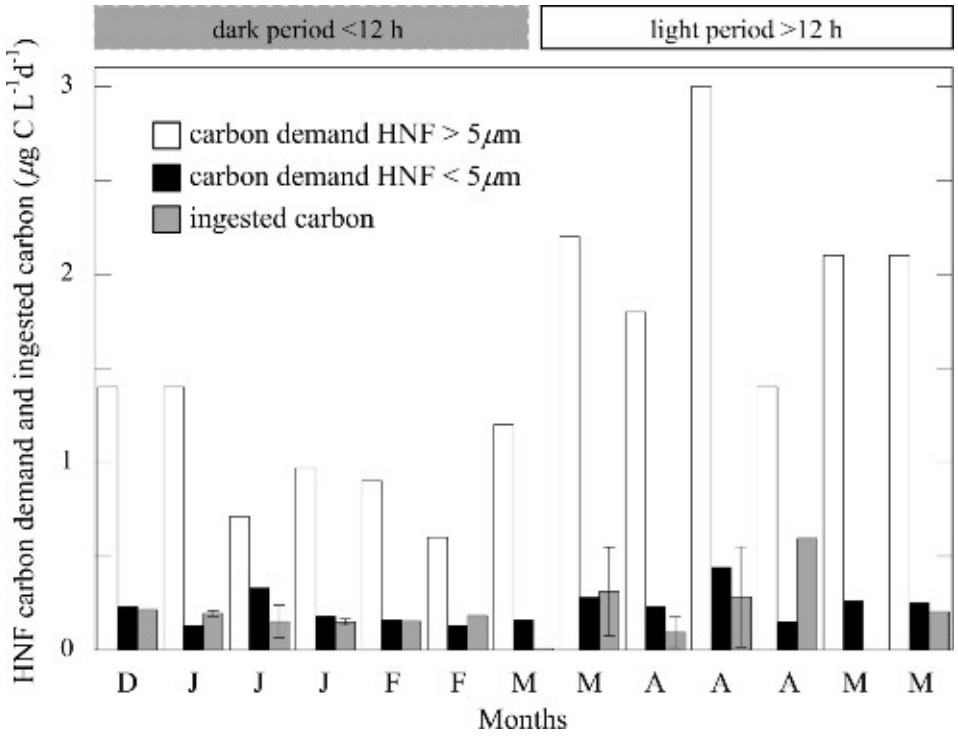

Fig. 5. Comparison between heterotrophic nanoflagellate carbon demand and carbon ingestion rates (G); error bars correspond to SD. Length of the light period is indicated in the upper part of the graph.

BB did not follow a defined pattern, and grazing on bacteria did not increase greatly in spring when bacterial production was stimulated. In winter bacterial production was likely controlled by grazing and in spring by resource supply. Finally, our estimations suggest that consumption of bacterial carbon alone would not fulfill the estimated carbon demand for growth of HNF >5 μm. Thus, these larger HNF probably feed on other cells such as *Micromonas* sp. in order to satisfy their carbon demands.

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