Different community structure and temperature optima of heterotrophic picoplankton in various regions of the Southern Ocean

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ABSTRACT: The temperature control of the growth of heterotrophic picoplankton was studied in the Southern Ocean along a transect between the Polar Front (49° S) and the shelf ice edge at 70° S in the austral summer. Growth was measured by thymidine and leucine incorporation applying the dual-label approach. Psychrotolerant or mesophilic communities with a growth optimum of >18°C were present in surface waters at the Polar Front at an *in situ* temperature of 4 to 5°C. Further south in surface waters of the Antarctic circumpolar current (ACC), in the marginal ice zone and at the shelf ice edge, psychrophilic communities with growth optima of 11 and 4 to 8°C, respectively, were present. Growth at the ambient temperature of ca 0°C or slightly below was reduced. By *in situ* hybridization with fluorescent rRNA-targeted oligonucleotide probes, between 63 and 96% of all DAPI-stainable picoplankton cells were identified as *Bacteria* whereas *Archaea* were never detected. Cells belonging to the *Cytophaga/Flavobacterium* cluster were most abundant from all bacterial groups tested (α -, β -, and γ -subclass of *Proteobacteria*, *Cytophaga/Flavobacterium* cluster) and comprised 20% of DAPI counts at the Polar Front and 40% in the ACC. In the marginal ice zone during a bloom of *Phaeocystis* sp. they even comprised 72%. γ -subclass proteobacteria accounted for <1% of DAPI counts at the Polar Front and for 7 to 9% further south. α -subclass proteobacteria never exceeded 1% and β -subclass proteobacteria were not detected at all. The results indicate that different communities of heterotrophic picoplankton established along the transect from the Polar Front south to the marginal ice zone as shown by the reduction of the temperature optima and coinciding with a change in the community structure and a pronounced dominance of bacteria of the *Cytophaga/Flavobacterium* cluster in the psychrophilic communities.

KEY WORDS: Heterotrophic picoplankton · Psychrophilic bacteria · Temperature · *In situ* hybridization · Oligonucleotide probes · Southern Ocean

INTRODUCTION

Heterotrophic picoplankton has been identified as one of the major trophic compartments in processing phytoplankton primary production in the world's oceans. As a mean for many regions including the Pacific, Atlantic, Indian and Southern Oceans, the ratios of picoplankton secondary production over primary production in the mixed layer range between 0.09 and 0.40 even though lower and higher ratios can

occur for short periods (Cota et al. 1990, Ducklow & Carlson 1992, Ducklow et al. 1993, Kirchman et al. 1993, Lochte et al. 1997). Interestingly, the ratios in the colder areas of the oceans such as in the subarctic Pacific and the Southern Polar Front are at the lower end, which supports the idea that temperature may limit picoplankton secondary production more than primary production at low temperature (Pomeroy & Deibel 1986). On the other hand, ratios of picoplankton secondary production over primary production in cold areas such as in the Antarctic circumpolar current (ACC) have also been reported to be elevated at low rates of primary production or because of a temporal

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uncoupling of these 2 processes (Cota et al. 1990, Billen & Becquevort 1991, Lochte et al. 1997).

A key question for understanding the relationship between picoplankton secondary production and primary production in the cold is how the heterotrophic picoplankton communities are adapted to the low temperature, in the sense whether psychrophilic, psychrotolerant or mesophilic communities sensu Morita (1975) establish and dominate the consumption of organic matter. This question was addressed in various studies which determined picoplankton secondary production or growth rates as a function of temperature. Most studies found that the optimum temperature of rates of production or growth was above the *in situ* temperature irrespective of whether psychrophilic or psychrotolerant communities were present (Azam et al. 1981, Hodson et al. 1981, Kirchman et al. 1993, Lochte et al. 1994).

An important implication of these findings leads to the hypothesis that regions with a pronounced difference in the temperature optimum of heterotrophic picoplankton growth exhibit a different community structure. This hypothesis, however, has not been tested. Various studies in different oceanic provinces provide some evidence for it. Using 16S rRNA-targeted oligonucleotide probes, differences in the composition of auto- and heterotrophic picoplankton in the Sargasso Sea, the coastal northwest Pacific and the Southern Ocean, regions with pronounced differences in the temperature regime, have been found (Giovannoni et al. 1990, DeLong et al. 1994). Very little, however, is known about the quantitative composition of the heterotrophic picoplankton in marine environments, even though a high qualitative genetic diversity of picoplankton phylotypes and a substantial fraction of *Archaea* in particular in cold environments have been found (Fuhrman et al. 1993, DeLong et al. 1994, Mullins et al. 1995, Massana et al. 1998, Murray et al. 1998). An ideal instrument for such studies is the *in situ* hybridization by rRNA-targeted fluorescent oligonucleotide probes (Amann et al. 1995, Glöckner et al. 1996), which recently has also been applied to coastal heterotrophic picoplankton (Ouverney & Fuhrman 1997).

We examined the temperature optima of heterotrophic picoplankton production at the Polar Front, in the ACC and in the marginal ice zone of the Southern Ocean. In the same regions and mostly at the same locations we also examined the composition of the heterotrophic picoplankton

by *in situ* hybridization with rRNA-targeted fluorescent oligonucleotide probes.

MATERIALS AND METHODS

This study was performed during the cruise ANT XIII/2 of the RV 'Polarstern' from Cape Town, South Africa, to the German Neumeyer base and back to Cape Town. Samples were collected at the Polar Front, in the ACC, the marginal ice zone, and directly at the shelf ice edge close to the Neumeyer base. For the dates and exact locations see Table 1. Samples south of the Polar Front, and samples for measurements of picoplankton growth at the Polar Front were taken from the surface with a bucket. Measurements of picoplankton growth started within 30 min of sampling. For *in situ* hybridization with fluorescent oligonucleotide probes samples at the Polar Front were taken with 12 l GoFlo bottles mounted on a rosette on 25 December 1995 at 50° 28.62' S, 08° 08' 82 E at 60 m and on 29 December 1995 at 49° 29.40' S, 11° 23.58' E at 20 and 40 m. From the bottles subsamples were withdrawn into acid-rinsed 1 l-polyethylene flasks, kept at *in situ* temperature and further processed within 2 h. Samples for *in situ* hybridization south of the Polar Front were also taken with a bucket from the surface.

To examine the temperature optima of heterotrophic picoplankton growth experiments were carried out in which this parameter was measured at various temperatures between –0.5 or 0 and 12 to 20°C. In the experiments at the Polar Front in early January the highest temperature tested was 12°C whereas on 19 January the range was extended to 20°C. The incubation temperatures were controlled carefully such that they

Table 1 Locations, dates of the experiments, *in situ* temperatures at the sampling sites in the Southern Ocean, numbers and *in situ* rates of biomass production of heterotrophic picoplankton. ACC: Antarctic circumpolar current; MIZ: marginal ice zone

Region	Location	Date (1996)	Temperature (°C)	Picoplankton Numbers (10 ⁵ ml ⁻¹)	Production (ng C l ⁻¹ h ⁻¹)
Polar Front	49° 42.15' S, 10° 18.32' E	3 Jan	4.92	4.88	56.75
	49° 45.00' S, 10° 30.04' E	4 Jan	4.11	5.29	47.67
	49° 23.50' S, 10° 58.06' E	19 Jan	5.04	8.11	145.55
	58° 56.52' S, 03° 58.44' E	9 Jan	0.11	1.51	10.30
MIZ	68° 50.57' S, 06° 01.09' E	11 Jan	0.12	3.88	36.11
Shelf ice edge	70° 29.89' S, 08° 15.47' E	13 Jan	–1.67	1.08	4.89

remained within $\pm 0.5^\circ\text{C}$ during the incubation time. Rates of biomass production and cell multiplication were determined by the incorporation of ^{14}C -leucine (Kirchman et al. 1985, Simon & Azam 1989) and ^3H -thymidine (TdR, Fuhrman & Azam 1980) using the dual-label approach (Chin-Leo & Kirchman 1988). Subsamples of 10 or 20 ml were withdrawn into clean polystyrene test tubes. Triplicates and a Formalin-killed control were labelled with ^{14}C -leucine (11.5 GBq mmol^{-1} , Amersham) and ^3H -thymidine (3 TBq mmol^{-1} , Amersham, diluted 1:5 with non-labelled TdR) at a final concentration of 10 nM each. Incubation at the various temperatures was in the dark and stopped after 4 to 6 h by adding Formalin (2 % final concentration). Prior to the addition of the radio-label the samples were preadjusted to the incubation temperatures for 30 min. After fixation samples were filtered onto 0.45 μm nitrocellulose filters (Sartorius), rinsed with ice-cold particle-free seawater and extracted with ice-cold 5 % trichloroacetic acid (TCA) for 5 min. After rinsing the extracted filters twice with ice-cold 5 % TCA, the filters were dissolved with ethylacetate and radioassayed by liquid scintillation counting. Biomass production was calculated from leucine incorporation rates by using a conversion factor of 3.0 kg C (mol leucine) $^{-1}$ (Simon & Azam 1989, Bjørnsen & Kuparinen 1991). To convert TdR incorporation into rates of cell multiplication a conversion factor of 1.4×10^{18} cells (mol TdR) $^{-1}$ (Bjørnsen & Kuparinen 1991) was used.

Temperature optima were defined as those temperatures or temperature ranges at which rates of biomass production and cell multiplication were highest or within 95 % of the highest rate. Responses of these rates to increasing temperatures were calculated on the basis of their respective Q_{10} values calculated as $Q_{10} = (K_1/K_2)^{10/(t_1-t_2)}$ (Sherr & Sherr 1996). K_1 and K_2 are rates at temperatures t_1 and t_2 (in $^\circ\text{C}$), where $t_1 > t_2$. The temperature ranges for which Q_{10} was calculated are given in Table 2.

In situ hybridization with fluorescent rRNA-targeted oligonucleotide probes was done according to Glöckner et al. (1996). The samples were filtered onto 0.2 μm Nuclepore filters and fixed by overlaying the filters with 3 ml of a freshly prepared, PBS-buffered (pH 7.2) 4 % paraformaldehyde solution for 30 min. Thereafter, the filters were washed with Milli-Q water and stored frozen at -20°C until hybridization after 4 mo. The hybridization protocol as described in Glöckner et al.

Table 2. Slopes of rates of heterotrophic picoplankton biomass production (PBP) and cell multiplication (PCM) versus temperature ($\text{ng C l}^{-1} \text{ h}^{-1} ^\circ\text{C}^{-1}$) and Q_{10} values for different temperature ranges in the Southern Ocean. ACC: Antarctic circumpolar current. For exact locations and dates see Table 1

Region		Range ($^\circ\text{C}$)	Slope		Q_{10}	
			PBP	PCM	PBP	PCM
Polar Front	3 Jan 96	0–11	7.03	1.97	5.2	3.8
	4 Jan 96	0–11	5.66	1.44	3.1	2.3
	19 Jan 96	0–20	27.52	7.79	32.5	18.5
		0–5	20.68 ^a	4.02	9.1 ^a	3.5
		10–20	20.63 ^b	9.77	4.0 ^b	4.7
ACC		0–11	3.67	1.88	3.7	2.7
		0–4	6.36	2.75	8.0	8.8
		4–11	2.75	1.57	2.3	2.1
Marginal ice zone		0–8	2.65	2.32	1.7	1.9
		0–2	6.68	3.38	2.5	2.4
		2–8	1.27	1.96	1.3	1.7
Shelf ice edge		–0.5–2	0.24	0.38	1.5	1.3
		2–4	1.58	1.28	8.2	34.7
		4–11	0.82	0.34	2.6	1.8

^a0–2.3 $^\circ\text{C}$; ^b10–15 $^\circ\text{C}$

(1996) was applied and the following group-specific oligonucleotide probes were used: EUB338 for *Bacteria* (Amann et al. 1990), ALF968 for α -subclass proteobacteria (Neef 1997), BET42a and GAM42a for β - and γ -subclass proteobacteria (Manz et al. 1992), and CF319a for members of the *Cytophaga/Flavobacteria* cluster (Manz et al. 1996). In addition, we applied the *Archaea*-specific probes ARCH344 and ARCH915 (Amann et al. 1995). The probes were linked to the N-hydroxysuccinimidester of the carbocyanine CY3 as the fluorochrome. The hybridization procedure includes a final staining with DAPI. Cells were viewed using an Axioplan epifluorescence microscope (Zeiss, Jena, Germany) and the filter sets Zeiss 01 for DAPI and Chroma HQ41007 (Chroma Tech. Corp. Brattleboro, VT, USA). For each sample 2 replicates were counted and 10 to 20 view fields with at least 500 cells per sample. All counts were corrected by subtracting the counts obtained with a negative control (NON338) which never exceeded 1 %.

RESULTS

The *in situ* temperature at the Polar Front ranged between 4 and 5 $^\circ\text{C}$ and decreased further south. In the ACC it was around 0 $^\circ\text{C}$ and dropped to below -1°C in the marginal ice zone close to the shelf ice edge (Table 1, Fig. 1). At the Polar Front the top 40 to 60 m (mixed layer) were well mixed as indicated by temperature profiles, numbers and production of heterotrophic picoplankton which agreed within 25 % between the surface and 60 m depth. At the Polar Front

Fig. 1. Transect of temperature at 5 m between the Polar Front and the shelf ice edge recorded between 8 and 12 January 1996. Temperature was measured online with a sensor on the RV 'Polarstern'. The arrows indicate the locations of the experiments at the Polar Front, the Antarctic circumpolar current (ACC), the marginal ice zone (MIZ), and the shelf ice edge (Shelf)

rates of heterotrophic picoplankton biomass production and cell multiplication continuously increased with temperature from 0 to 11 and 20°C (Fig. 2A). The 2 experiments in early January showed similar re-

sponses to increasing temperature whereas on 19 January growth increased much faster with temperature. On 19 January picoplankton numbers and *in situ* rates of biomass production were much higher than in early January (Table 1) even though the vertical distribution of numbers and production rates in the upper 60 m were not different in early and on 19 January. Interestingly, the patterns of temperature response in the ACC and the marginal ice zone were quite different despite similar *in situ* temperatures (Table 1, Fig. 2B). In the ACC picoplankton growth had an optimum at 11°C and decreased above this temperature. Between 0 and 4°C increase with temperature was much stronger than between 4 and 11°C. In fact, rates at 4 and 7°C were not statistically different both for biomass production and cell multiplication (Student's *t*-test, $p < 0.01$). In the marginal ice zone rates of biomass production were highest in the temperature range of 4 to 8°C whereas those of cell multiplication were in the range of 8 to 11°C (Fig. 2B). The rate of increase in biomass production, which was between 0 and 2.4°C at this location, was similar to that, between 0 and 4°C, in the ACC whereas that of cell multiplication rates was even higher (Table 2). At the shelf ice edge picoplankton growth between -0.5 and 2°C remained constant at very low rates as the slopes of increase of picoplankton growth within this temperature range were not statistically different from zero (Table 2). Growth only

Fig. 2. Biomass production and cell multiplication of heterotrophic picoplankton $\pm$ standard deviation at various temperatures at (A) the Polar Front and (B) in the Antarctic circumpolar current (ACC), the marginal ice zone, and at the shelf ice edge. Arrows indicate the *in situ* temperature. If standard deviation is not visible, it is smaller than the symbols. For the exact locations of the experiments and *in situ* temperatures see Table 1

increased above 2°C and was highest in the range of 11 to 17°C (Fig. 2B).

Q_{10} values, calculated for the entire temperature ranges and various sections with fairly continuous increase, varied from 1.3 to 34.7 (Table 2). They were highest at the Polar Front for the entire range tested and at the shelf ice edge for the range between 2 and 4°C. The lowest values, 1.3 to 1.9, were recorded in the marginal ice zone and at the shelf ice edge for the ranges between 2 and 8, and -0.5 and 2°C, respectively. Q_{10} values of biomass production and cell multiplication agreed particularly well in the ACC and the marginal ice zone. In 67% of the experiments, though, the Q_{10} of biomass production was higher than that of cell multiplication. This finding, that biomass production increases more with temperature than cell division, is consistent with similar observations by Shiah & Ducklow (1994) and Tibbles (1997).

In situ hybridization with oligonucleotide probes showed a high detection rate in all samples (Figs. 3 & 4). Between 61 and 96% of all picoplankton cells stainable with DAPI hybridized with the probe EUB338. The probes specific for *Archaea* ARCH344 and ARCH915 did not give any positive signals in the samples examined. Samples for *in situ* hybridization at the Polar Front were not collected at the exact locations of the temperature shift up experiments but at stations very close to them. Because all results of *in situ* hybridization at the Polar Front were in close agree-

ment, we only include 1 example of 40 m of the station 49° 29.40' S, 11° 23.40' E in Fig. 3. At this station 24% (range of all 3 stations: 19 to 24%) of the DAPI-stainable cells ($9.0 \times 10^8 \text{ l}^{-1}$) were identified as belonging to the *Cytophaga/Flavobacterium* cluster, 11% to α -subclass proteobacteria (range of all 3 stations: 4 to 11%) and 1% to γ -subclass proteobacteria (Fig. 3). Also, at the other 2 stations γ -subclass proteobacteria comprised not more than 1% of DAPI-stainable cells. β -subclass proteobacteria were not detected at the Polar Front or at other locations. In fact, we did not find any signals of the probe BET42a in any of the samples. South of the Polar Front the proportion of bacteria belonging to the *Cytophaga/Flavobacterium* cluster continuously increased. In the ACC 40% of the DAPI-stainable cells were identified as members of this cluster. In the marginal ice zone, where we collected samples at the southern edge of an intense bloom of *Phaeocystis* sp. with chlorophyll concentrations of 1.5 to 2.0 $\mu\text{g l}^{-1}$ (U. Bathmann unpubl. results), as much as 72% of the DAPI-stainable cells belonged to the *Cytophaga/Flavobacterium* cluster (Figs. 3 & 4). Also, the proportion of γ -subclass proteobacteria increased in the ACC and the marginal ice zone but did not exceed 9%. The proportion of α -subclass proteobacteria remained fairly similar in all areas sampled. At the Polar Front, in the ACC and the marginal ice zone 47, 65, and 91%, respectively, of the cells detected with the probe EUB338 could be characterized with the group-specific probes applied.

Fig. 3. Community structure of the heterotrophic picoplankton as determined by *in situ* hybridization with rRNA-targeted fluorescent oligonucleotide probes at the Polar Front (40 m), in the Antarctic circumpolar current (ACC, surface) and the marginal ice zone (surface; mean values $\pm$ standard deviation). Probe data are given as percent of cells detectable after staining with DAPI. For the exact locations of the experiments and dates see Table 1. Specifications of the probes are as follows: EUB338 for *Bacteria*; CF319a for the *Cytophaga/Flavobacterium* cluster; ALF968 for α -subclass proteobacteria; BET42a for β -subclass proteobacteria; and GAM42a for γ -subclass proteobacteria

DISCUSSION

The heterotrophic picoplankton in the various regions of the Southern Ocean exhibited pronounced differences with respect to growth control by temperature. The optimum temperature continuously decreased from 20°C at the Polar Front to 11°C in the ACC and 4 to 8°C in the marginal ice zone. Even though at the shelf ice edge lowest rates of biomass production and cell multiplication were measured at *in situ* temperature, the optimum temperature was in the range of 11 to 17°C and thus higher than in the marginal ice zone. Hence, the results indicate that at the Polar Front facultatively psychrophilic, psychrotolerant or mesophilic picoplankton communities *sensu* Morita (1975) dominated, whereas further south obligately psychrophilic communities with different temperature optima were present. In all regions, the optimum temperature for bacterial growth was above the *in situ* temperature.

Several other studies carried out in cold oceanic regions also found that the optimum growth temperature of the heterotrophic picoplankton was above the

Fig. 4. Colour micrographs of picoplankton cells stained by DAPI (A, C, E) and by *in situ* hybridization with CY3-labeled rRNA-targeted oligonucleotide probes (B, D, F, G, H). (A, B) Polar Front; (C, D) ACC; (E, F, G, H) marginal ice zone with *Phaeocystis* cells. (A to F) show identical viewfields stained by DAPI and the probe EUB338. (G) shows *Cytophaga/Flavobacteria* stained by probe CF319a and (H) shows α -subclass proteobacteria stained by probe ALF968

in situ temperature (Azam et al. 1981, Hodson et al. 1981, Kirchman et al. 1993, Lochte et al. 1994). Whereas this notion is not surprising when mesophilic or psychrotolerant communities are present, it may be unexpected that psychrophilic communities also always exhibit a higher optimum than *in situ* growth temperatures. A possible explanation may be that psychrophilic microbes do not optimize growth or biomass production rates at the fairly low *in situ* temperatures but the growth yield. Morita (1975) reported that for one psychrophilic bacterial isolate grown at the usually high substrate concentrations applied in bacterial pure culture studies the growth yield was maximized below the optimum temperature for growth. Whether this notion is a general phenomenon of psychrophiles and also true at low substrate concentrations such as under ambient conditions *in situ* remains to be examined.

Interestingly, the temperature optimum of biomass production and cell multiplication of heterotrophic picoplankton at the Polar Front was not below 20°C, and thus at least 8 to 12°C higher than further south in the ACC and the marginal ice zone, even though the *in situ* temperatures in all 3 regions were 5°C and below. Unfortunately, we limited the temperature range tested in early January at the Polar Front from 0 to 11°C but we assume that the optimum was not different than 2 wk later. Kirchman et al. (1993) in the subarctic Pacific also found that heterotrophic picoplankton growth exhibited a temperature optimum of 20°C whereas the *in situ* temperature was only 6°C. Studies covering seasons with greatly varying *in situ* temperatures in the Chesapeake Bay, the Baltic Sea and small freshwater lakes also reported that the optimum growth temperature of heterotrophic picoplankton was fairly constant between 20 and 25°C and was independent of the *in situ* temperature except for a short period in summer at highest *in situ* temperatures (Lovell & Konopka 1985, Autio 1992, Morris & Lewis 1992, Shiah & Ducklow 1994, Coveney & Wetzel 1995). Simon & Wünsch (1998), however, found that in the large and deep Lake Constance, Germany, *in situ* and optimum growth temperatures of heterotrophic picoplankton in the upper 10 m usually differed by not more than 5°C despite *in situ* temperatures ranging from 5 to 23°C. In accordance with these reports and the present study we hypothesize that temperature-adapted heterotrophic picoplankton communities establish only when thermal conditions for mesophilic

communities are favorable or, in the case of psychrophilic communities, in systems with little large-scale mixing of different water masses and/or a residence time of the water masses far exceeding the generation time of the ambient picoplankton. Such conditions are given in the ACC and the marginal ice zone, and in large lakes with a pronounced pelagic zone such as Lake Constance but not at the Polar Front or in other frontal systems including estuaries and most small lakes.

A conclusion from the cited studies is that temperature can control growth of heterotrophic picoplankton in 2 different ways, either directly when growth is a positive function of temperature, or indirectly by selecting the establishment of a community well adapted to the ambient temperature. Both types of control have specific implications for the transfer of organic matter in the food web which are different at different *in situ* temperatures, e.g. in cold (<5°C) or warmer environments. We note that, in addition, availability of and preferences for organic substrates and inorganic nutrients may also control the growth of heterotrophic picoplankton (Morris & Lewis 1992, Coveney & Wetzel 1995, Schweitzer & Simon 1995). Further, the temperature control of phytoplankton, proto- and mesozooplankton growth relative to that of heterotrophic picoplankton may also be important in controlling the transfer of organic matter in the food web at various temperature ranges. Pomeroy & Deibel (1986) found evidence that below 4°C picoplankton secondary production (PSP) is more limited by temperature than primary production (PP), reducing the ratio of PSP/PP. Examining substrate utilization of bacteria at low temperatures in more detail, Wiebe et al. (1992, 1993) found that psychrotolerant bacterial isolates grew faster at high than at low substrate concentrations at the minimum growth temperature but still at substantially lower growth rates than at 15°C or above. On the other hand, Harder & Veldkamp (1971) reported that a psychrophilic bacterial isolate with a growth optimum at 14.5°C grew faster between -2 and +10°C both at low and at high substrate concentrations than a mesophilic isolate with a growth optimum at 30°C. These findings suggest that mesophilic and psychrotolerant bacteria are more substrate-limited at low than at high temperatures as compared to psychrophilic bacteria. They imply that if psychrotolerant bacteria are prominent in heterotrophic picoplankton communities substrate limitation at low temperature

becomes relatively more important than if psychrophilic bacteria dominate. The relatively low ratios of PSP/PP in cold oceans of <0.15 (Cota et al. 1990, Kirchman et al. 1993, Lochte et al. 1997) further suggest that growth and production of heterotrophic picoplankton communities in these environments are limited more by temperature than PP. This effect will be more pronounced if mesophilic bacteria dominate the community such as at the Polar Front (Lochte et al. 1997, this study) or in the subarctic Pacific (Kirchman et al. 1993). We further hypothesize that if a picoplankton community that is well adapted to the ambient temperature, whether psychrophilic or mesophilic, is established and the transfer processes of DOM are unchanged, the ratio of PSP/PP should be the same in cold as well as in temperate and warm environments.

In situ hybridization with fluorescent rRNA-targeted oligonucleotide probes is a powerful tool for characterizing microbial communities in aquatic environments. So far, this method has been successfully applied to characterize bacterial communities in lakes, on lake snow aggregates, on activated sludge and in coastal waters (Wagner et al. 1993, Alfreider et al. 1996, Glöckner et al. 1996, Weiss et al. 1996, Ouverney & Fuhrman 1997, Pernthaler et al. 1998). Our results demonstrate that this method is also suitable for characterizing picoplankton assemblages in oceanic environments such as the Southern Ocean. Between 63 and 96% of the DAPI-stainable cells were detected as *Bacteria* with the highest proportion in the marginal ice zone. From the Polar Front to the marginal ice zone the proportion of cells of the *Cytophaga/Flavobacterium* cluster increased from 20 to 72% of DAPI counts. Interestingly, the highest proportion of *Cytophaga/Flavobacterium* was detected in a *Phaeocystis* bloom in the marginal ice zone. Obviously, the mucus- and substrate-rich environment of this colonial algae, which often dominates the phytoplankton in the early austral spring (Kang & Fryxell 1993, Putt et al. 1994, Karentz & Spero 1995), favors the establishment of homogeneous blooms of psychrophilic *Cytophaga/Flavobacterium* populations.

The changes in the structure of the bacterial community along the transect coincided with the decrease of the optimum temperature of picoplankton growth (see above), indicating that the psychrophilic communities south of the Polar Front were increasingly dominated by bacteria of the *Cytophaga/Flavobacterium* cluster. At the Polar Front, samples for the temperature shift-up experiments and the analysis of the structure of the picoplankton community were not taken on the same day and at the same depth. The 3 samples from 20, 40 and 60 m depths which we used to analyze community structure, however, exhibited only minor variations, suggesting that they were representative of the mixed

layer and the given seasonal situation at the Polar Front including presumably also on 19 January, for which we had only data on the temperature shift-up experiments and total picoplankton numbers and growth. Growth was substantially higher than 2 to 3 wk before, which may explain the different temperature response of the picoplankton growth as compared to early January. We also assume that the temperature response of the picoplankton growth between the surface and the bottom of the mixed layer was not different and thus our findings with surface samples representative for the picoplankton community of the entire mixed layer.

Our findings on the composition of the picoplankton community are consistent with observations that the obligately psychrophilic bacteria isolated in various types of ice in the Southern Ocean are frequently members of the *Cytophaga/Flavobacterium* cluster (Helmke & Weyland 1995, Bowman et al. 1997, E. Helmke pers. comm). These findings also show that in the austral summer the heterotrophic picoplankton communities in surface waters of the Southern Ocean south of the Polar Front consist predominantly of bacteria of known phylogenetic lineages. Microbes of lineages newly discovered, e.g. members of the *Archaea*, have been detected in other oceanic regions (Giovannoni et al. 1990, Fuhrman et al. 1993, Mullins et al. 1995) and also in the Southern Ocean, but mainly in winter and in deeper water layers and with decreasing proportions towards summer and phytoplankton blooms (DeLong et al. 1994, Massana et al. 1998, Murray et al. 1998). *Archaea* obviously did not play a prominent role in the surface waters of the regions of the Southern Ocean we studied during the austral summer.

An interesting result was that we did not detect any signal of β -subclass proteobacteria in any of the samples. No signal of this phylogenetic analyzing 16S rRNA phylotypes in oceanic samples lineage has been detected in other studies either (Giovannoni et al. 1990, Fuhrman et al. 1993, Mullins et al. 1995). It is noteworthy that in bacterial assemblages in limnetic systems including bacterioplankton, lake snow aggregated and activated sludge (Wagner et al. 1993, Alfreider et al. 1996, Glöckner et al. 1996, Weiss et al. 1996, Pernthaler et al. 1998) β -subclass proteobacteria often comprise large proportions and even dominate.

In summary, we have shown that the growth of bacterial communities at the Polar Front, in the ACC, the marginal ice zone and at the shelf ice edge exhibited pronounced differences in the temperature optimum. At the Polar Front facultatively psychrophilic or mesophilic bacterial assemblages dominated and further south obligate psychrophiles. *In situ* hybridization with rRNA-targeted fluorescent oligonucleotide probes

showed that the psychrophilic picoplankton had a different composition and was dominated by bacteria of the *Cytophaga/Flavobacterium* cluster whereas *Proteobacteria* and *Archaea* played only a minor role.

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