

Different coupling of dissolved amino acid, protein, and carbohydrate turnover to heterotrophic picoplankton production in the Southern Ocean in austral summer and fall

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Abstract

We assessed growth dynamics of heterotrophic picoplankton and concentrations and turnover of dissolved protein, amino acids, and neutral monosaccharides in the Atlantic sector of the Southern Ocean in austral summer (December–January) and fall (March–May). Phytoplankton biomass (chlorophyll *a*) and biomass production of heterotrophic picoplankton in summer was twice as high as in fall. This difference was also reflected in protein turnover rate constants and in concentrations of dissolved combined neutral polysaccharides (DCCHO). Turnover rate constants of dissolved free amino acids (DFAA) were in the same range in both seasons, but turnover rate constants of glucose were higher in fall as compared to summer. In summer, dissolved protein was the major substrate for growth of heterotrophic picoplankton, followed by DFAA and dissolved free neutral monosaccharides (DFCHO). During summer, heterotrophic picoplankton production was closely correlated to concentrations and incorporation of dissolved protein. In fall, heterotrophic picoplankton production was only significantly correlated to glucose turnover rate constants. The latter were also inversely correlated to DCCHO concentrations in fall. The reduced supply of organic substrates by phytoplankton in fall not only resulted in an equal reduction of heterotrophic picoplankton production but also in a shift of the supply in dissolved protein, DFAA, and DFCHO to heterotrophic picoplankton. Dissolved protein was the major substrate for heterotrophic picoplankton growth in summer, but in fall, when supply of dissolved protein was reduced, DFAA and DFCHO were relatively more important substrates.

There is ample evidence that dissolved amino acids and dissolved carbohydrates are the major substrates for growth of heterotrophic picoplankton in pelagic ecosystems. This evidence is predominantly based on studying the picoplankton consumption of dissolved free amino acids (DFAA) and dissolved combined amino acids (DCAA), dissolved protein, and dissolved free neutral monosaccharides (DFCHO; Münster 1993; Rosenstock and Simon 1993, 2001). Most such studies have been carried out in coastal systems and focused either on DFAA, DCAA, and protein (e.g., Coffin 1989; Keil and Kirchman 1993; Meon and Amon 2004) or on DFCHO (Bunte and Simon 1999; Meon and Amon 2004). These studies indicate that protein and DFAA are more important substrates than glucose, but the relative significance between both former substrates varies. Far fewer studies have been carried out in oceanic systems, which are less productive than coastal systems. In tropical and subtropical oceanic systems, such as the central Pacific, the Gulf of Mexico, and the Red Sea, glucose uptake accounted for <10–30% of the carbon demand for heterotrophic picoplankton biomass production (Rich et al. 1996; Skoog et al. 1999; Grossart and Simon 2002).

Similar fractions were reported from the north Pacific and polar oceans (Rich et al. 1997; Kirchman et al. 2001; Skoog et al. 2002). In the Arctic Ocean, uptake of DFAA has been reported to be two to fivefold higher than that of DFCHO (Rich et al. 1997). In the only oceanic study, carried out in the Sargasso Sea, that assessed uptake of all three substrates simultaneously, protein was found to be more important than DFAA, and glucose was the least important substrate (Keil and Kirchman 1999). Because of the small number of studies, we still have a fragmentary understanding of the relative significance of these substrates for growth of heterotrophic picoplankton in oceanic regions and how uptake is coupled to the availability of the substrates, i.e., concentrations.

The Southern Ocean occupies around 10% of the world's ocean surface, and variables affecting the biosphere globally such as climate warming and stratospheric ozone depletion have a special effect on this cold and sensitive biome. This is one reason why biogeochemical processes in this region have been studied extensively during the last two decades. Phytoplankton primary production and biomass production and respiration of heterotrophic picoplankton are quantitatively probably the most important biogeochemical processes in the carbon cycle in the pelagic zone of the Southern Ocean (e.g., Lochte et al. 1997; Ducklow et al. 2001; Pedrós-Alió et al. 2002). Several comprehensive investigations of dynamics of heterotrophic picoplankton and their relationship to phytoplankton biomass and other controlling factors have been carried out in various regions of the Southern Ocean (e.g., Sullivan et al. 1990; Bird and Karl 1999; Simon et al. 2004). It is surprising, though, that information on concentration dynamics and the relative significance of the major labile substrates for growth of heterotrophic picoplankton is still scarce. Whereas several

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studies exist that determined turnover rate constants of DFAA and glucose (e.g., Cota et al. 1990; Sullivan et al. 1990; Kirchman et al. 2001), to our knowledge, only one study exists which measured uptake of glucose in relation to heterotrophic picoplankton production and its coupling to concentrations of dissolved combined neutral monosaccharides (DCCCHO; Kirchman et al. 2001).

We studied concentrations, turnover rate constants, and uptake of DFAA, dissolved protein, and DFCHO and concentrations of DCAA and DCCHO in relation to growth dynamics of heterotrophic picoplankton and to chlorophyll *a* (Chl *a*) as a proxy for phytoplankton biomass in the upper 100 m in the Atlantic sector of the Southern Ocean in the austral summer and fall.

Material and methods

Study area, sampling, and chlorophyll—The study was carried out on board RV *Polarstern* during cruise ANT-XIII/2 (04 December 1995 to 24 January 1996) in the austral summer and cruise ANT-XVI/3 (18 March to 10 May 1999) in the austral fall in the Atlantic sector of the Southern Ocean (Table 1). Nineteen stations of the summer cruise and 15 stations of the fall cruise, located in the polar frontal zone, the Antarctic circumpolar current, the Weddell Sea, and the coastal current under pack ice close to the shelf ice are included in this study. The study regions are rather similar to those investigated by Simon et al. (2004) and shown in fig. 1 of that publication. Samples between 20 and 100 m were collected with 12-liter Niskin bottles mounted on a General Oceanics Rosette sampler equipped with a Neil Brown Mark III CTD. Subsamples were withdrawn into acid-rinsed 1-liter polyethylene bottles, kept at in situ temperature ($\pm 1^\circ\text{C}$) and were further processed within 1 h. Chl *a* was determined after extraction in 90% acetone fluorometrically according to Tremblay et al. (2002).

Heterotrophic picoplankton abundance—Subsamples of 50 mL were fixed with 2% formalin and stored at 2°C until further processing within 2 d. Depending on the abundance, 3–5 mL were stained with 4,6-diamidinophenyleindole (DAPI, 1 mg 100 mL⁻¹) for 5 min, filtered onto black 0.2- μm Nuclepore membranes, and kept at 2°C until enumeration by epifluorescence microscopy (Porter and Feig 1980) with a Nikon microscope (Labophot 2A) on shipboard within 1 week.

Heterotrophic picoplankton production—Production rates were determined from the incorporation of ¹⁴C-leucine according to Simon and Azam (1989) and as described in detail in Simon et al. (2004). Briefly, ¹⁴C-leucine (specific activity 11.5 GBq mmol⁻¹, Amersham [ANT-XIII/2]; 10.8 GBq mmol⁻¹, Hartmann Analytik [ANT-XVI/3]) was added to 10-mL subsamples at a final concentration of 10 nmol L⁻¹ in triplicates and a formalin-killed control. Incubation was at in situ temperature ($\pm 1^\circ\text{C}$) in the dark and stopped after 5 to 7 h by adding formalin (1% final concentration). After fixation, samples were filtered onto 0.2- μm nitrocellulose filters (25-mm diameter, Sartorius),

Table 1. Station numbers, latitude, longitude, region (ACC, Antarctic circumpolar current; CC, coastal current; PFZ, polar frontal zone; Wed, Weddell Sea) and date of sampling during cruises ANT XIII/2 (summer) and ANT XVI/3 (fall) with RV *Polarstern*.

Station no.	Latitude (S)	Longitude	Region	Date
Summer				
5	50°11.1'	05°30.8' W	PFZ	09 Dec 95
7	57°19.0'	02°05.3' W	ACC	11 Dec 95
9	53°59.9'	00°06.2' E	ACC	22 Dec 95
10	50°28.8'	08°09.1' E	PFZ	25 Dec 95
11	49°54.8'	10°17.6' E	PFZ	27 Dec 95
12	49°29.3'	11°23.3' E	PFZ	29 Dec 95
13	49°32.4'	11°51.6' E	PFZ	29 Dec 95
14	50°18.1'	11°31.6' E	PFZ	30 Dec 95
15	50°41.8'	11°31.0' E	PFZ	30 Dec 95
16	51°06.1'	10°17.4' E	PFZ	30 Dec 95
17	51°30.1'	10°17.5' E	PFZ	31 Dec 95
18	50°42.0'	09°34.1' E	PFZ	05 Jan 96
19	49°54.0'	09°34.1' E	PFZ	05 Jan 96
20	49°30.0'	10°17.8' E	PFZ	06 Jan 96
22	49°59.5'	10°17.3' E	PFZ	06 Jan 96
25	50°18.0'	10°17.5' E	PFZ	07 Jan 96
29	50°42.0'	10°17.5' E	PFZ	07 Jan 96
32	49°53.8'	11°33.1' E	PFZ	20 Jan 96
33	49°42.0'	11°31.5' E	PFZ	20 Jan 96
Fall				
153	52°00.6'	20°00.0' E	PFZ	25 Mar 99
154	49°51.6'	20°00.0' E	PFZ	26 Mar 99
156	48°49.8'	20°00.0' E	PFZ	27 Mar 99
157	49°20.4'	20°00.0' E	PFZ	28 Mar 99
163	48°51.1'	20°04.0' E	PFZ	04 Apr 99
165	49°20.3'	20°00.3' E	PFZ	05 Apr 99
166	49°36.8'	20°01.8' E	PFZ	05 Apr 99
169	60°00.0'	20°31.8' E	Wed	09 Apr 99
174	70°10.7'	06°51.6' W	CC	16 Apr 99
182	70°13.8'	06°07.9' W	CC	19 Apr 99
185	66°53.6'	00°00.0' E	Wed	21 Apr 99
197	51°54.0'	19°58.8' E	PFZ	02 May 99
200	50°00.6'	20°00.0' E	PFZ	02 May 99
201	49°29.4'	20°01.2' E	PFZ	04 May 99
203	48°30.6'	20°00.0' E	PFZ	04 May 99

rinsed with ice-cold particle-free seawater, and extracted with ice-cold 5% trichloroacetic acid. The filters were radioassayed by liquid scintillation counting. The coefficient of variation (CV, standard deviation/mean) of the triplicate measurements was usually <10%. Biomass production was calculated from leucine incorporation rates by using a conversion factor of 1.5 kg C (mol leucine)⁻¹, assuming no intracellular isotope dilution (Simon and Azam 1989).

Turnover and incorporation rates of DFAA and DFCHO—Turnover rate constants of DFAA and glucose were determined by measuring the incorporation of a mixture of 16 ³H-DFAA (mean specific activity 1.97 GBq mat C, TRK440, Amersham) and ³H-glucose (specific activity 429.0 GBq mmol⁻¹, Amersham) by heterotrophic picoplankton. Each radiolabel was added to a separate set of triplicate 10-mL subsamples and a formalin-killed control at a final

concentration of 0.1 nmol L^{-1} of DFAA and glucose. Incubations were further processed as for heterotrophic picoplankton production. Turnover rate constants of DFAA and glucose were calculated as the ratio of the radioactivity incorporated per hour over the radioactivity added. Hence, they are rate constants assessing the turnover of these substrates fuelling biosynthesis and do not include respiration. Incorporation rates of DFAA and DFCHO were determined by multiplying the respective turnover rate constants with measured DFAA and DFCHO concentrations (*see below*) and assuming $50 \text{ g C (mol amino acid)}^{-1}$ and $72 \text{ g C (mol monosaccharide)}^{-1}$. The CV of the triplicate measurements was usually $<15\%$.

Turnover, incorporation, and concentration of dissolved protein—Turnover rate constants of dissolved protein were determined by measuring the incorporation of a ^{14}C -algal protein (specific activity $1.63 \text{ GBq mmol}^{-1}$, Amersham). This custom-made protein, extracted from *Anacystis nidulans*, served as a model substrate for labile proteins of a wide range in molecular weight and typical under natural conditions. The radiolabel was added in triplicates to 10-mL subsamples and a formalin-killed control at 2 nmol L^{-1} final concentration (amino acid equivalent). Incubations were further processed as for heterotrophic picoplankton production. For further details see Rosenstock and Simon (2001). Protein turnover rate constants were calculated as the ratio of the radioactivity incorporated per hour over the radioactivity added and represent net turnover rate constants, not considering respiration. Incorporation rates were calculated by multiplying the turnover rate constants with concentrations of the dissolved protein and assuming $50 \text{ g C (mol amino acid in protein)}^{-1}$. The latter were determined from incorporation kinetics according to Wright and Hobbie (1965) and calculated from the derived $K_t + S_n$ values (K_t , substrate concentration at half maximum incorporation rate; S_n , in situ substrate concentration). Therefore, incorporation of ^{14}C -protein was measured at four concentrations (1, 20, 50, and 100 nmol L^{-1} final concentration). Incubation and further processing was done as described for measurements of heterotrophic picoplankton production, and S_n was calculated as 50% of $K_t + S_n$ (Billen 1991; Keil and Kirchman 1993). The CV of the triplicate measurements was $<10\%$.

Concentrations of dissolved amino acids and dissolved neutral sugars—Concentrations of DFAA were measured by high performance liquid chromatography (HPLC) after precolumn derivatization with *ortho*-phthaldialdehyde as described previously (Rosenstock and Simon 2001). Special care was taken to avoid contaminations from sampling to analysis by always working with Latex gloves and, using only ultraclean chemicals and combusted glass ware. The detection limit for individual amino acids was 0.5 nmol L^{-1} , and the mean coefficient of variation (CV, standard deviation) of replicate analyses was 5%. TDAA were hydrolyzed in 6 mol L^{-1} HCl for 1 h at 155°C . α -Amino butyric acid served as internal standard and was added to the samples prior to hydrolysis to account for

possible losses due to the handling. To account for possible contamination during handling, three blanks per batch of samples were treated and analyzed in the same way as the samples and subtracted from the concentration of the samples. Concentrations of TDAA, analyzed as DFAA after hydrolysis, are given as monomer equivalents. Concentrations of dissolved free neutral sugars (DFCHO) were determined by HPLC with a Dionex 500 system and pulsed amperometric detection according to Mopper et al. (1992). The preparation of samples and eluants is described in detail by Bunte and Simon (1999). To avoid a negative peak due to oxygen in the chromatogram, we used a CarboPac PA-10 instead of a PA-1 column. Hydrolysis of total dissolved neutral sugars (TDCHO) was done with 0.09 mol L^{-1} HCl at 100°C for 20 h. Samples were desalted by ion exchange chromatography as described by Rich et al. (1996). We separated eight monosaccharides (arabinose, fructose, fucose, galactose, glucose, mannose, rhamnose, xylose) at a solvent concentration of 20 mmol L^{-1} NaOH and a column temperature of 20°C . Concentrations were calculated relative to an external standard consisting of a mixture of the eight monosaccharides analyzed. To account for possible contamination during handling, three blanks per batch of samples were always treated and analyzed in the same way as the samples and subtracted from the concentration of the samples. Concentrations of TDCHO, analyzed as DFCHO after hydrolysis, are given as monomer equivalents. The detection limit of individual monosaccharides was 5 nmol L^{-1} and the CV of triplicate measurements 10%.

Results

During the summer cruise, temperatures in the surface layer ranged between -0.2°C at the southernmost station (Sta. 7) and $2.5\text{--}4.5^\circ\text{C}$ at the polar frontal stations. During the fall cruise, temperatures in the surface layer of the polar frontal region decreased from 5.9°C at the northern fringe around 49°S to 1.9°C at 52°S . At 60°S (Sta. 169) and south of 66° in the pack ice (Sta. 174 and 182) temperatures were 0.45 and -1.75°C , respectively.

During both seasons, there was a substantial accumulation of phytoplankton biomass, (Chl *a* concentrations) in the polar frontal region as compared to further south. In summer and fall, Chl *a* reached up to 148 and $49 \text{ mg Chl } a \text{ m}^{-2}$ ($0\text{--}100 \text{ m}$) in the polar frontal region, respectively, as compared to 33 and $25 \text{ mg Chl } a \text{ m}^{-2}$ further south (Figs. 1 and 2). The overall mean concentrations in summer were twice as high as in fall (Table 2). During both cruises, highest Chl *a* concentrations usually did not occur in the upper 20 m of the water column but below, between 40 and 100 m depth (Figs. 3 and 4). In summer, highest Chl *a* concentrations at individual stations in the polar frontal region ranged from 0.3 to $1.85 \text{ } \mu\text{g Chl } a \text{ L}^{-1}$, and in fall from 0.2 to $0.75 \text{ } \mu\text{g Chl } a \text{ L}^{-1}$. Data for the Weddell Sea (Stas. 169, 185) from the fall cruise are not available, and the highest Chl *a* concentrations in the coastal current below pack ice (Stas. 174 and 182) were 0.08 and $0.17 \text{ } \mu\text{g Chl } a \text{ L}^{-1}$.

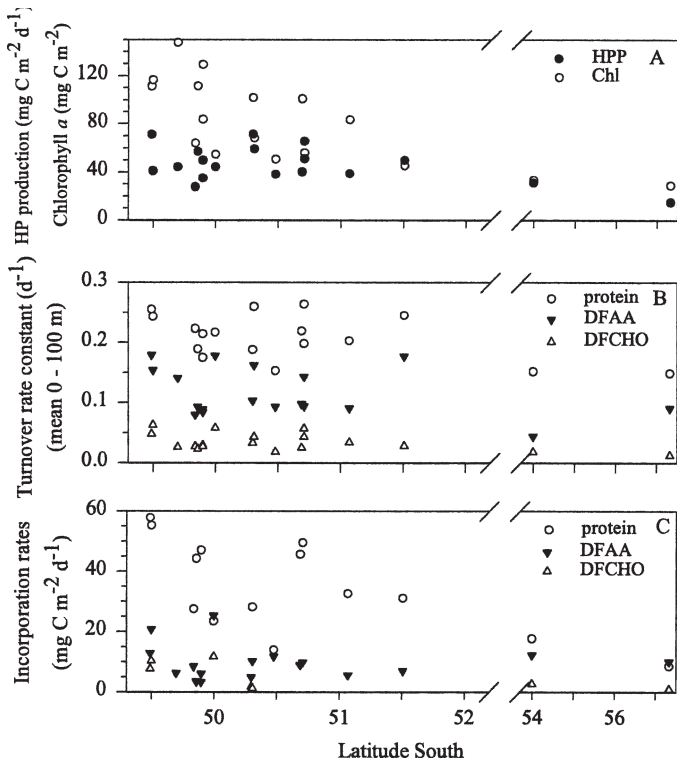


Fig. 1. (A) Integrated rates of heterotrophic picoplankton (HP) production, chlorophyll *a*, (B) mean turnover rate constants of dissolved protein, DFAA, and DFCHO (central panel), and (C) integrated incorporation rates for the upper 100 m in the Atlantic sector of the Southern Ocean in austral summer.

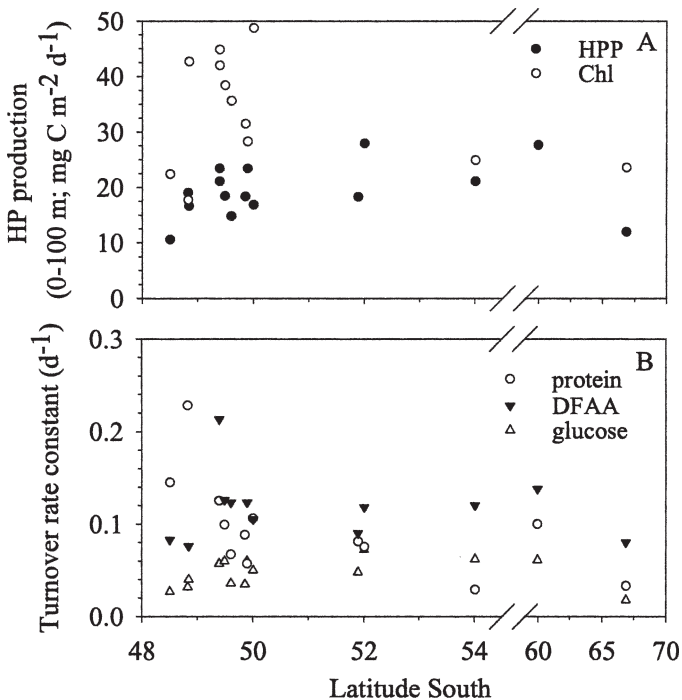


Fig. 2. (A) Integrated rates of heterotrophic picoplankton (HP) production and (B) mean turnover rate constants of dissolved protein, DFAA, and DFCHO in the Atlantic sector of the Southern Ocean in austral fall.

Heterotrophic picoplankton—Heterotrophic picoplankton numbers varied from 1.3×10^8 to 7.3×10^8 cells L⁻¹ in summer with lower values in the Antarctic circumpolar current (ACC), and from 1.6×10^8 to 5.9×10^8 cells L⁻¹ in fall without any systematic patterns among the various regions. The overall means of both cruises were not significantly different (Table 2). Vertical patterns were rather variable during both seasons (Figs. 3 and 4). Numbers did not correlate to any other parameter assessed.

The accumulation of phytoplankton biomass in the polar frontal region was reflected by slightly enhanced integrated rates of heterotrophic picoplankton production in summer, but in fall integrated rates remained rather similar in all regions (Figs. 1 and 2). Volumetric rates of heterotrophic picoplankton production during the summer cruise varied between 2 and 40 ng C L⁻¹ h⁻¹, with lowest values at Stas. 5 and 7 at the beginning of the cruise and highest values at Stas. 12, 13, 18, and 25. There was quite a variability among individual stations and vertically (Fig. 3). During the fall cruise, rates of heterotrophic picoplankton production ranged from 1 to 18.9 ng C L⁻¹ h⁻¹ without any systematic vertical pattern (Fig. 4) and with an overall mean of 7.9 ± 3.1 ng C L⁻¹ h⁻¹, half of that of the summer cruise (Table 2).

Substrate concentrations—Concentrations of DCAA, DFAA, and dissolved protein were only determined during the summer cruise. DCAA concentrations ranged from 180 to 585 nmol L⁻¹ with low values (<300 nmol L⁻¹) at Stas. 5, 7, 12, and 14 and high values (>400 nmol L⁻¹) at Stas. 9, 11, 18, and 32. Vertical patterns were rather variable and did not covary with any other parameter assessed. The mol% composition of DCAA was fairly constant, and glycine + threonine, alanine, aspartate, and serine, on average, constituted 22, 16, 12, and 11 mol%, respectively. The other amino acids constituted <8 mol%, each. DFAA concentrations also varied greatly, between 8 and 400 nmol L⁻¹. The overall mean was 53.0 ± 61.4 nmol L⁻¹ (Table 2) and thus 16.4% of DCAA concentrations. Concentrations >100 nmol L⁻¹ occurred only when rates of heterotrophic picoplankton production were <16 ng C L⁻¹ h⁻¹, whereas at production rates >17 ng C L⁻¹ h⁻¹ concentrations remained <60 nmol L⁻¹ (Fig. 5). As with DCAA, there were little variations in the mol% composition of DFAA. Serine, glycine + threonine, glutamate, and alanine, on average, constituted 23, 21, 11, and 10 mol%, respectively, and the other amino acids <8 mol%, each. Concentrations of dissolved protein varied less than those of DCAA and DFAA, from 13 to 145 nmol L⁻¹ with lowest values (<40 nmol L⁻¹) at Stas. 7 and 10 and an overall mean not significantly different from that of DFAA (Table 2). Concentrations of DCAA did not exhibit any significant correlation with other parameters assessed. DFAA concentrations were significantly positively correlated to Chl *a*, and protein concentrations to heterotrophic picoplankton production ($p < 0.01$; Table 3, Fig. 5).

Concentrations of DCCHO during the summer cruise, determined only at 8 of the 18 stations, varied greatly among the stations, but also with depth, from 160 to

Table 2. Mean values of Chl *a*, numbers (HP numbers), and biomass production of heterotrophic picoplankton (HP production), concentrations of dissolved free amino acids (DFAA) and dissolved combined amino acids (DCAA), protein, free neutral (DFCHO) and combined monosaccharides (DCCHO), turnover rate constants of dissolved proteins, DFAA, and DFCHO, incorporation rates of DFAA, protein, and DFCHO of all pooled samples between 20 and 100 m depth during cruises ANT XIII/2 and ANT XVI/3. *n*, number of samples, nd, not determined, bd, below detection limit.

Parameter	ANT XIII/2 summer	<i>n</i>	ANT XVI/3 fall	<i>n</i>
Chlorophyll <i>a</i> ($\mu\text{g L}^{-1}$)	0.72 ± 0.53	83	0.35 ± 0.13	55
HP numbers (10^8 L^{-1})	3.43 ± 1.42	87	3.26 ± 0.84	64
HP production ($\text{ng C L}^{-1} \text{ h}^{-1}$)	16.4 ± 10.7	87	7.9 ± 3.1	64
DFAA (nmol L^{-1})	53.0 ± 61.4	84	nd	
DCAA (nmol L^{-1})	323.4 ± 124.9	74	nd	
Protein (nmol L^{-1})	57.4 ± 39.8	50	nd	
DFCHO (nmol L^{-1})	31.6 ± 18.1	29	bd	47
DCCHO (nmol L^{-1})	378.3 ± 353.4	22	195.1 ± 156.6	47
DFAA turnover rate constant (d^{-1})	0.117 ± 0.064	71	0.086 ± 0.068	60
Protein turnover rate constant (d^{-1})	0.209 ± 0.070	59	0.112 ± 0.052	56
Glucose turnover rate constant (d^{-1})	0.034 ± 0.017	71	0.048 ± 0.020	76
DFAA inc. rate ($\text{ng C L}^{-1} \text{ h}^{-1}$)	7.7 ± 6.2	65	nd	
(%HP production)	52.4 ± 45.8		nd	
Protein inc. rate ($\text{ng C L}^{-1} \text{ h}^{-1}$)	24.9 ± 19.5	50	nd	
(%HP production)	137.6 ± 71.6		nd	
DFCHO inc. rate ($\text{ng C L}^{-1} \text{ h}^{-1}$)	4.2 ± 4.1	28	nd	
(%HP production)	26.2 ± 30.4		nd	

1,500 nmol L^{-1} . Values $>600 \text{ nmol L}^{-1}$ occurred at Stas. 7, 20, and 25, and there was a general trend of decreasing concentrations with depth (Fig. 3). In fall, concentrations

were systematically lower and ranged from 35 to 620 nmol L^{-1} . Values $>300 \text{ nmol L}^{-1}$ were recorded toward the end of the cruise at Stas. 197, 200, and 201 at the polar front, in the Weddell Sea (Sta. 169), and in the coastal current (Stas. 174 and 182; Fig. 4). Particularly low values,

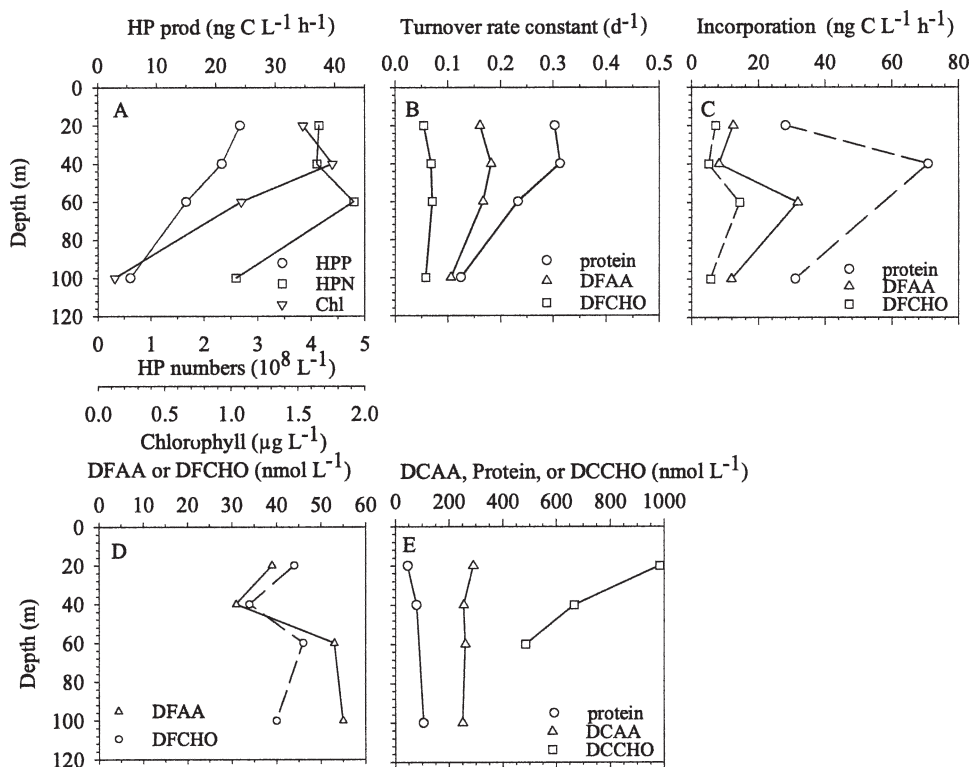


Fig. 3. (A) Depth profiles of heterotrophic picoplankton production (HPP), heterotrophic picoplankton numbers (HPN), Chl *a*, (B) turnover rate constants, and (C) incorporation rates of dissolved protein, DFAA, and DFCHO, (D) concentrations of DFAA and DFCHO, and (E) DCAA, protein, and DCCHO at Sta. 20 ($49^{\circ}30.0' \text{ S}$, $10^{\circ}17.8' \text{ E}$) in the polar front in the Atlantic sector of the Southern Ocean in austral summer.

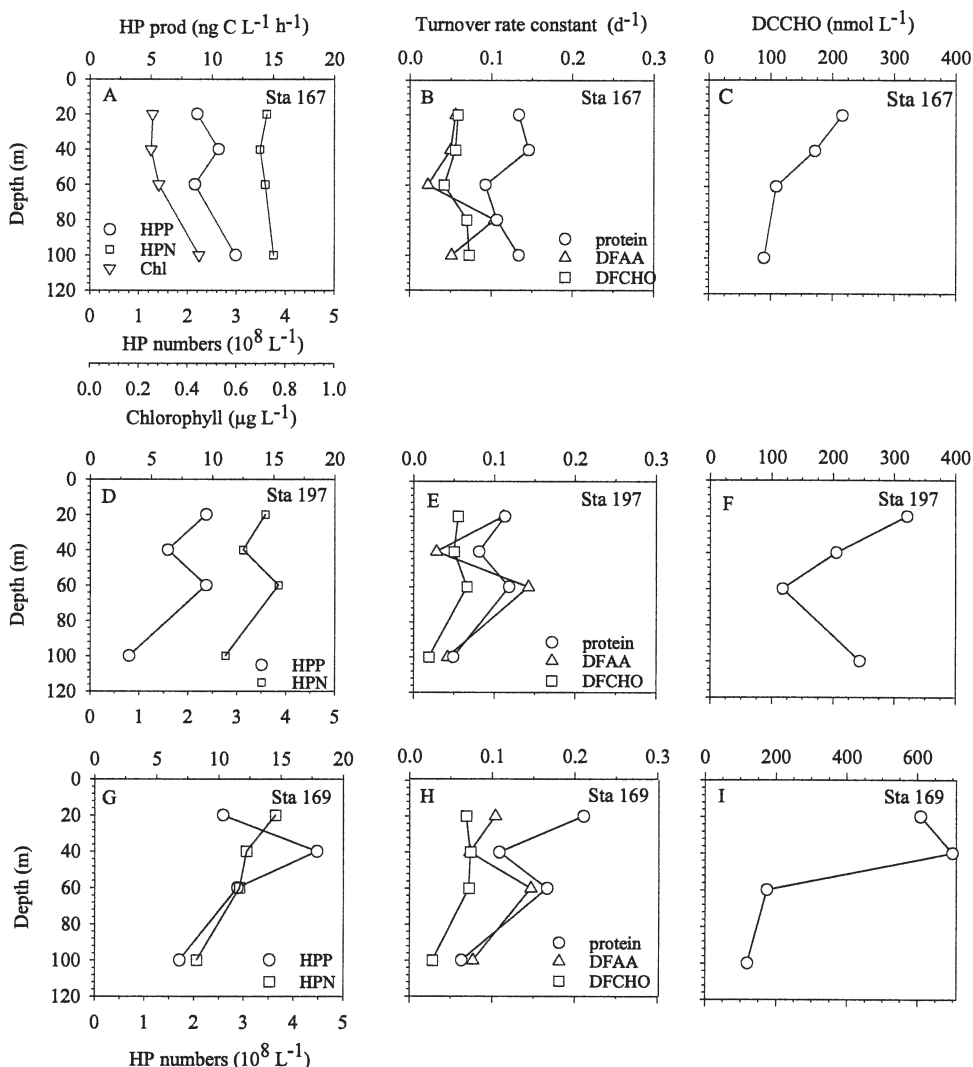


Fig. 4. (A, D, G) Depth profiles of heterotrophic picoplankton (HP) production, heterotrophic picoplankton numbers, and Chl *a*. (B, E, H) Turnover rate constants of dissolved protein, DFAA, and DFCHO. (C, F, I) Concentrations of DCCHO at Stas. 167 (polar frontal zone), 197 (polar frontal zone), and 169 (Weddell Sea) in the Atlantic sector of the Southern Ocean in austral fall. For exact locations of the stations see Table 1).

<100 nmol L⁻¹, occurred early during the cruise at Stas. 153, 154, and 157 at the polar front. In contrast to the summer cruise, there was no trend of decreasing concentrations with depth. The overall mean concentration was 195.1 ± 156.6 nmol L⁻¹, half of that of the summer cruise (Table 2). Glucose always dominated DCCHO and constituted 45–73 mol%, followed by mannose with 14–24 mol% and galactose with 9–22 mol%. The other monosaccharides detectable, fucose, rhamnose, and arabinose, always constituted <8 mol% each. Concentrations of DFCHO during the summer cruise varied from 6 to 70 nmol L⁻¹ without any clearcut patterns, neither vertically nor regionally. Glucose dominated the DFCHO pool by 40–80 mol%. The other monosaccharides, arabinose, fucose, galactose, mannose, and rhamnose, constituted <15 mol% each and often were below the detection limit (5 nmol L⁻¹). During the fall cruise, DFCHO concentra-

tions remained below the detection limit. Concentrations of DCCHO were significantly positively correlated to heterotrophic picoplankton production and Chl *a* during the summer cruise ($p < 0.01$; Table 3, Fig. 6).

Substrate turnover—Protein turnover rate constants during the summer cruise ranged from 0.074 to 0.417 d⁻¹, with an overall mean of 0.209 ± 0.070 d⁻¹ (Table 2). During the fall cruise, turnover rate constants were roughly 50% lower than during the summer cruise, ranging from 0.044 to 0.288 d⁻¹ and with a mean of 0.112 ± 0.052 d⁻¹ (Figs. 2 and 4, Table 2). In the Weddell Sea and the coastal current, rate constants were generally lower than in the polar frontal region. In the latter region, rate constants were lower early in the cruise (Stas. 154, 156, 157). Protein turnover rate constants were significantly positively correlated to heterotrophic picoplankton pro-

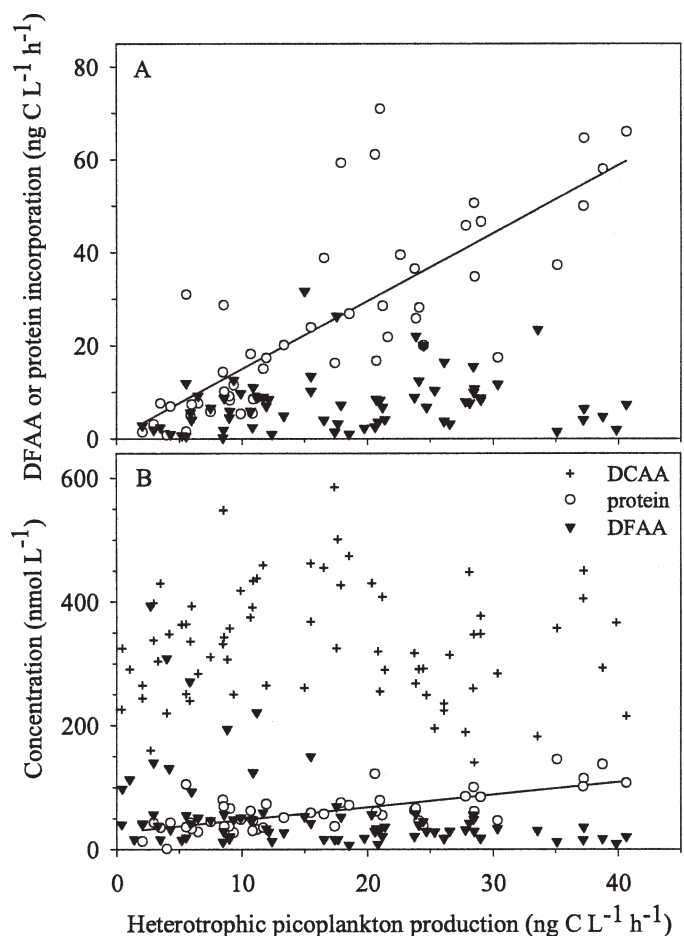


Fig. 5. (A) Scatter plot of heterotrophic picoplankton production and incorporation of dissolved protein and DFAA. (B) Concentrations of dissolved protein, DCAA, and DFAA in the Atlantic sector of the Southern Ocean in austral summer. Also given are regression lines of heterotrophic picoplankton production and protein incorporation (panel A) and concentration (panel B). For regression statistics see Table 3.

duction and to DFAA turnover rate constants during the summer cruise but not during the fall cruise ($p < 0.01$; Table 3).

DFAA turnover rate constants ranged from 0.013 to 0.303 d^{-1} during the summer cruise and from 0.017 to

0.392 d^{-1} during the fall cruise, but the mean value of the summer cruises was significantly higher than that of the fall cruise (t -test, $p < 0.01$; Table 2). DFAA turnover rate constants were significantly positively correlated to heterotrophic picoplankton production and to protein turnover rate constants during the summer cruise ($p < 0.01$; Table 3).

In contrast to turnover rate constants of protein and DFAA, those of glucose were enhanced during the fall cruise as compared to the summer cruise. Mean values of the former cruise were 1.4-fold higher (Table 2), despite nearly similar ranges of the values during both cruises, 0.007–0.081 d^{-1} and 0.008–0.100 d^{-1} , respectively. During the fall cruise glucose turnover rate constants exhibited a significantly positive correlation to heterotrophic picoplankton production (Fig. 6) and a significantly negative correlation to DCCHO concentrations of Stas. 153–157 ($p < 0.01$; Table 3, Fig. 7).

The direct comparison of the substrate turnover rate constants exhibited distinct differences among the three parameters and between both cruises. Turnover rate constants of protein were systematically higher than those of DFAA during the summer cruise but not during the fall cruise (Fig. 8). Turnover rate constants of protein were always higher than those of glucose, except in two cases, but ratios of paired values were higher during the summer as compared to the fall cruise (Fig. 8). DFAA turnover rate constants were higher than glucose turnover rate constants during the summer cruise. During the fall cruise, however, turnover rate constants of glucose were higher than those of DFAA in 25% of all cases, and in 42% of all cases higher than these ratios of the summer cruise (Fig. 8).

Substrate incorporation—Because substrate concentrations were only determined during the summer cruise, data on substrate incorporation are only available for this cruise. Protein incorporation rates ranged from 0.80 to 70.9 $\text{ng C L}^{-1} \text{h}^{-1}$. Highest values occurred in the polar frontal region (Fig. 1). Protein incorporation was significantly and closely correlated to heterotrophic picoplankton production ($p < 0.01$; Table 3, Fig. 5). It explained the majority of the C demand for heterotrophic picoplankton production, as indicated by the high ratio of protein incorporation over heterotrophic picoplankton production, often exceeding 100% (Table 2, Figs. 3, 5). DFAA in-

Table 3. Regression coefficients of linear regression statistics ($p < 0.01$) between the listed variables for the cruises in summer (Ant XIII/2) and fall (ANT XVI/3). HPP, heterotrophic picoplankton production. ns, nonsignificant correlations; — not available.

Variable A	Variable B	Summer r^2	Fall r^2
Chlorophyll	DFAA concentration	0.63	—
Chlorophyll	DCCHO concentration	0.25	ns
HPP	Protein concentration	0.49	—
HPP	Protein incorporation	0.62	—
HPP	DFAA turnover rate constant	0.25	ns
HPP	DCCHO concentration	0.25	ns
HPP	Glucose turnover rate constant	ns	0.58
Glucose turnover rate constant	DCCHO concentration	—	-0.35*
Protein turnover rate constant	DFAA turnover rate constant	0.41	ns

* Stas. 153, 154, 156, 157 only.

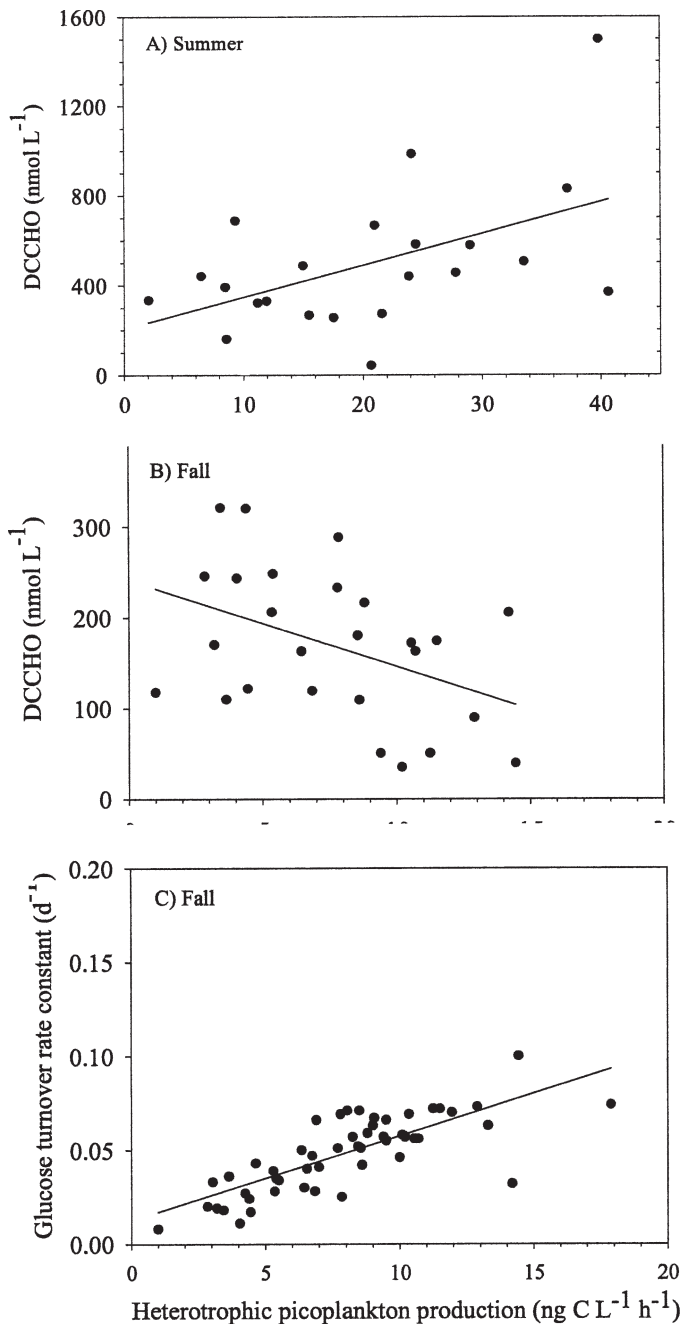


Fig. 6. Linear regression analysis ($p < 0.01$) of heterotrophic picoplankton production and concentrations of DCCHO in (A) austral summer, (B) fall. (C) Glucose turnover rate constants in fall in the Atlantic sector of the Southern Ocean. Note the different scales of the x -axes in summer and fall.

corporation rates were highly variable and ranged from 0.20 to 31.7 $\text{ng C L}^{-1} \text{h}^{-1}$. In most cases, incorporation rates of DFAA were lower than those of protein (Figs. 3, 5) and the mean value was only 31% of that of protein incorporation (Table 2). At low rates of heterotrophic picoplankton production, however, incorporation rates of DFAA were in the same range as those of protein (Fig. 5). DFCHO incorporation rates ranged from 0.10 to 14.3 $\text{ng C L}^{-1} \text{h}^{-1}$ and were always substantially lower than those of

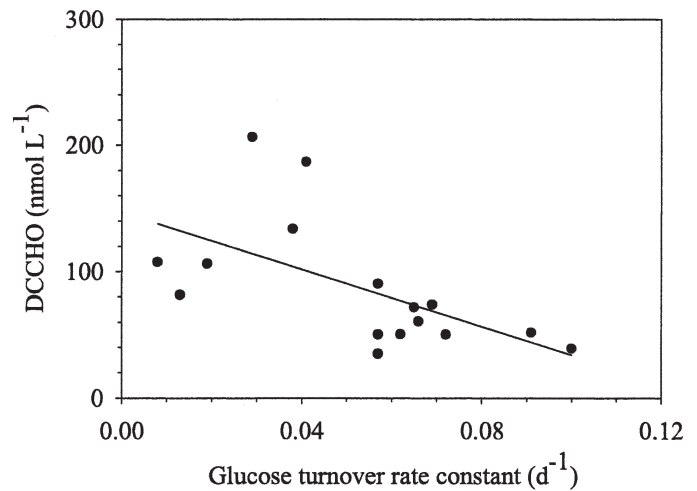


Fig. 7. Linear regression analysis ($p < 0.01$) of glucose turnover rate constants and concentrations of DCCHO of Stas. 153, 154, 156, and 157 in austral fall in the Atlantic sector of the Southern Ocean.

protein and DFAA. On average they explained 26.2% of the C demand for heterotrophic picoplankton production, and thus only 50% of that of DFAA incorporation (Table 2).

Discussion

Seasonality of substrate dynamics in the Southern Ocean—Our results show that phytoplankton biomass in the polar frontal region of the Southern Ocean in the austral summer was twice as high as in fall. This twofold difference was also reflected by heterotrophic picoplankton production, protein and DFAA turnover rate constants, and DCCHO concentrations. In contrast, glucose turnover rate constants were enhanced in fall as compared to summer. These findings indicate that the reduced supply of organic substrates by phytoplankton in fall resulted not only in an equal reduction of heterotrophic picoplankton production but also in a shift of the supply in dissolved protein, DFAA, and DFCHO to heterotrophic picoplankton. Dissolved protein was the major substrate for heterotrophic picoplankton growth in summer at an enhanced standing stock of phytoplankton and high rates of primary production (Tremblay et al. 2002). In fall, when supply of dissolved protein was reduced, DFAA and DFCHO became relatively more important substrates for growth of heterotrophic picoplankton. The enhanced significance of DFAA appeared to be mainly due to the reduced significance of dissolved protein. A further indication may be that DFAA incorporation in summer remained rather independent of heterotrophic picoplankton production (Fig. 5). The enhanced significance of dissolved carbohydrates as substrates in fall was demonstrated by enhanced glucose turnover rate constants. Further, there was a negative relationship between DCCHO concentrations and heterotrophic picoplankton production in fall as compared to a positive one in summer, indicating a net reduction of the DCCHO pool in fall at

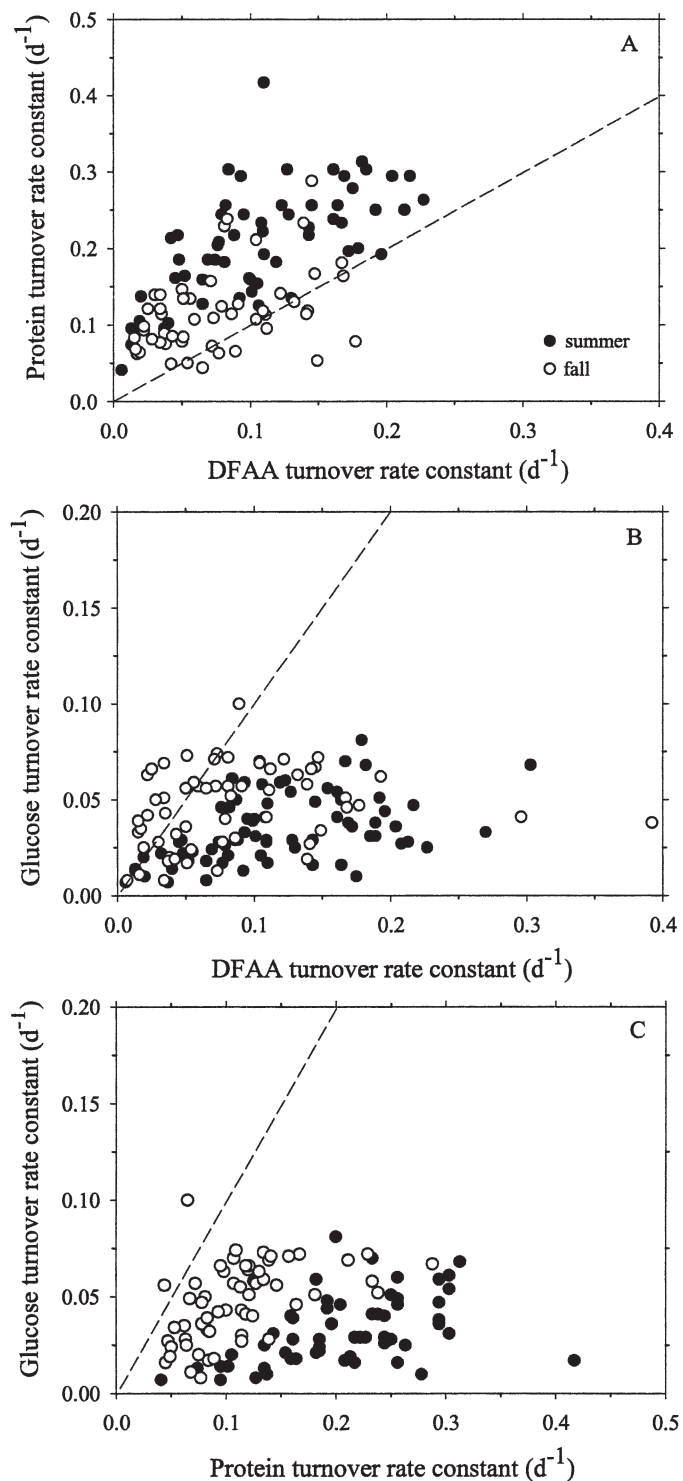


Fig. 8. (A) Scatter plot of turnover rate constants of DFAA and protein, (B) of DFAA and glucose, and (C) of protein and glucose in summer and fall in the Atlantic sector of the Southern Ocean. The dashed line indicates the 1:1 line.

elevated rates of heterotrophic picoplankton production. A further indication of the close coupling between carbohydrate turnover and heterotrophic picoplankton production is the fact that DFCHO concentrations in fall remained

below the detection limit (5 nmol L^{-1}). In addition, turnover rate constants of glucose were positively correlated to heterotrophic picoplankton production in fall, whereas turnover rate constants of the other substrates did not exhibit significant correlations to heterotrophic picoplankton production in this season.

Unfortunately, we did not measure concentrations of dissolved proteins and DFAA during the fall cruise. Hence we cannot calculate incorporation rates of these substrates and thus substantiate our notion of the relative significance of the three substrates for this season. Nevertheless the correlation analysis discussed above provides good evidence for the relative shift in the significance of the three substrates in fall as compared to summer. A further difference may be that the turnover of dissolved carbohydrates in fall may not be the result of uptake via DFCHO (mainly glucose) but rather may be caused by the fact that hydrolysis and uptake of DCCHO are closely coupled, involving uptake systems (porines) for oligosaccharides.

Our results thus indicate that dissolved protein was the preferred substrate for heterotrophic picoplankton, yielding the highest production rates. This scenario occurred during the summer cruise, when phytoplankton biomass was twice as high as in fall. DFAAs were less important than protein but preferred against DFCHO, which appeared to be mainly a supplementary substrate, supporting only half as much of heterotrophic picoplankton production as DFAA. Carbohydrates are only a carbon source, in contrast to amino acids which supply both carbon and nitrogen. Hence, carbohydrates appear to be mainly used if the availability of amino acids and protein does not meet the demand, and presumably as an energy source. In the only other study, which assessed bacterial use of these three substrates, carried out in the Sargasso Sea, protein was also preferred relative to DFAA and DFCHO (Keil and Kirchman 1999). However, turnover and uptake rates of these substrates were higher than in our study, presumably because of the completely different climatic and trophic situation of both ecosystems. These findings appear to be a general feature of oceanic and possibly of other pelagic systems in which phytoplankton primary production is the main substrate source for heterotrophic picoplankton.

We want to note that we only assessed net incorporation of the three substrates into biomass, not taking into account the fractions that were respired. Respiration and its relative fraction of total consumption both of amino acids and glucose certainly varied between both substrate classes and between summer and fall. Thus, gross turnover rate constants of both substrate classes were not only higher than the net turnover rate constants we assessed but presumably were more variable between both seasons. However, because of the various significant regressions between net turnover rate constants and incorporation rates of these substrates and other parameters (Table 3), we assume that our findings are still valid.

Substrate dynamics in oceanic systems—Evidence for a relative preference of DFCHO as substrates for heterotrophic picoplankton at low organic matter supply

is given by results from a study in the equatorial Pacific (Rich et al. 1996). These authors assessed turnover of DFCHO during periods of high (August–September) and low primary production and bacterioplankton production (February–March) and found that turnover and uptake rates of DFCHO were substantially higher when bacterioplankton production was low. During this period DFCHO uptake supported 32–51% of bacterioplankton production, whereas it supported only 4–23% when bacterioplankton production was high. This study, however, does not allow us to draw any conclusions on the relative significance of other substrates.

Seasonal differences in the relative significance of glucose uptake for heterotrophic picoplankton growth and in turnover rate constants of DFAA and glucose have also been found in the Ross Sea and the polar frontal region north of the Ross Sea (Kirchman et al. 2001). Rates of heterotrophic picoplankton production and turnover of DFAA and glucose in austral summer and fall reported by these authors were in the same range as in our study. In the Ross Sea, however, turnover rate constants of DFAA were systematically higher than those of glucose in both seasons, but in the polar frontal region north of the Ross Sea turnover rate constants of both substrates were more similar and without pronounced seasonal differences. As in our study, in some cases at the polar front turnover rate constants of glucose exceeded those of DFAA. In contrast to our study, DFCHO supported only <1–6% of heterotrophic picoplankton production in the Ross Sea and 4–11% in the polar frontal region and thus was substantially less important than we found.

Concentrations of DFAA in our study were in the same range as in other oceanic systems such as the Arctic Ocean (Rich et al. 1997; Meon and Amon 2004) and subtropical systems (Keil and Kirchman 1999; Grossart and Simon 2002). This was also true for concentrations of DFCHO, for which comparable data are also available from subpolar and polar systems (Rich et al. 1997; Kirchman et al. 2001; Meon and Amon 2004) and from subtropical systems (e.g., Rich et al. 1996; Keil and Kirchman 1999; Skoog et al. 1999).

The DFAA turnover rate constants of our study were in the same range as in the central Arctic Ocean (Rich et al. 1997) but lower than in the coastal Arctic Ocean (Kara Sea), Sargasso Sea, and the Red Sea (Keil and Kirchman 1999; Grossart and Simon 2002; Meon and Amon 2004), indicating that the trophic state and/or temperature enhance the turnover of DFAA. This notion appears also to be true for glucose because the turnover rate constants of this substrate in the Southern Ocean (this study; Kirchman et al. 2001) are lower than in warmer oceanic systems such as the Pacific (Rich et al. 1996; Skoog et al. 2002), the Sargasso Sea and Gulf of Mexico (Keil and Kirchman 1999; Skoog et al. 1999), and the Red Sea (Grossart and Simon 2002). They are also lower than in the Arctic Ocean (Rich et al. 1997; Meon and Amon 2004), suggesting that supply by dissolved carbohydrates (DFCHO, DCCHO) and coupling to use by heterotrophic picoplankton is different from the Southern Ocean, possibly because of higher inputs of carbohydrate-rich organic matter from large rivers discharging into the Arctic Ocean.

Budget of substrate incorporation and implications for constraining heterotrophic picoplankton production—Incorporation of protein often exceeded the carbon demand of heterotrophic picoplankton production in our study, and, as a mean, protein supported 138% of heterotrophic picoplankton production (Table 2). Incorporation of all three substrates together, as a mean for the summer cruise, accounted for roughly 200% of the demand for heterotrophic picoplankton production. Also other studies found that the combined incorporation of DFAA and DFCHO exceeded 100% of heterotrophic picoplankton production (Rich et al. 1997; Grossart and Simon 2002). Possible reasons for this mismatch is contamination during substrate analysis, which may be a problem in particular at very low concentrations. We do not have any indication of serious contamination problems with our samples, but cannot rule out that several rather high concentrations of DFAA might have been contaminated. Another possibility may be an underestimation of production rates by heterotrophic picoplankton, when conservative conversion factors are applied. We applied the most conservative conversion factor for the leucine method, assuming no intracellular isotope dilution (ID). In empirical studies, intracellular ID of leucine by a factor of two has been found in oceanic systems (Simon and Azam 1989; Simon 1991). Applying a twofold intracellular ID would double our production rates, thus accommodating incorporation of all three substrates assessed with rates of heterotrophic picoplankton production. Hence, the substrate incorporation rates we determined do not appear to be unrealistically high and are also in the same ranges as in other oceanic systems (*see above*). Our picoplankton production rates, in fact, leave some room for adjustment to a higher level. Empirical conversion factors for the leucine method determined in the Southern Ocean range from no intracellular ID (Ducklow et al. 2000; Pedrós-Alió et al. 2002) to a factor of 2 (Bjørnsen and Kuparinen 1991).

In conclusion, we have shown that differences in phytoplankton standing stock in the Southern Ocean in austral summer and fall are not only reflected in differences in heterotrophic picoplankton production but also lead to a shift in substrate supply to heterotrophic picoplankton. Whereas in summer at high phytoplankton standing stock, dissolved proteins are the major substrates for heterotrophic picoplankton growth and DFAA, and in particular dissolved carbohydrates become increasingly important in fall at reduced phytoplankton standing stock, supporting only lower rates of heterotrophic picoplankton production. This scenario appears to occur also in oceanic systems in other climatic regions even though other effects such as temperature may further contribute to these differences.

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