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Heterotrophic bacterial activity and fluxes of dissolved free amino acids and glucose in the Arctic rivers Ob, Yenisei and the adjacent Kara Sea

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ABSTRACT: Various parameters of bacterial activity were measured in the Kara Sea system (Arctic) including the freshwater endmembers of the rivers Ob and Yenisei during August/September 2001. Average bacterial production in surface water was highest in the rivers (13.5 µg C l^{-1} d^{-1}) and decreased towards the estuaries (5.8 µg C l^{-1} d^{-1}) and the open Kara Sea (2.4 µg C l^{-1} d^{-1}). Bacterial production in the salinity gradient was significantly correlated to chlorophyll *a* concentrations ($r = 0.78$, $p < 0.001$), indicating a tight coupling between primary production and bacterial growth. Similar to bacterial production mean turnover-rate constants of dissolved free amino acids (DFAA) and glucose decreased from the rivers (1.5 to 1.9 d^{-1}) towards the Kara Sea (0.4 d^{-1}). In combination with low *in situ* concentrations of DFAA (<50 nM) and glucose (<10 nM) and the immediate stimulation of bacterial growth upon addition of glucose in incubation experiments this suggests that bacteria were limited by the availability of organic carbon and that the supply of substrates governed the distribution pattern of bacterial growth. Mean uptake of DFAA contributed more to bacterial production (24 to >100%) than glucose uptake (<10%). Addition of filtered water from the Yenisei to untreated water from the Kara Sea caused no increase in bacterial production relative to the controls, indicating that riverine dissolved organic material is not the primary source of labile compounds for the bacterial community of the Kara Sea. Based on a bacterial growth efficiency of 27% that was derived from bacterial production and respiration measurements, our estimates for the bacterial carbon demand confine the export of surplus primary production from the Kara Sea to the central Arctic.

KEY WORDS: Bacteria · Arctic Ocean · Dissolved free amino acids · Glucose · Riverine dissolved organic material · Bacterial carbon demand · Bacterial growth efficiency · Arctic rivers · Kara Sea

INTRODUCTION

Heterotrophic bacteria are the major sink for dissolved organic carbon (DOC) in the ocean, with an estimated 40 to 60% of primary production being channeled through their metabolism (Cole et al. 1988, Ducklow & Carlson 1992, Nagata 2000). Consequently, heterotrophic bacteria constitute a key component of the oceanic carbon cycle, shaping both the amount and composition of the DOC pool (e.g. Williams 2000, Amon et al. 2001, Meon & Kirchman 2001). Thanks to extensive research efforts in the last 2 decades, our knowledge about bacterial actvity in various coastal and oceanic regions has increased considerably (Ducklow 2000). However, there are still some blank spots on the map. Among these, the Arctic Ocean with its extended shelf areas definitely belongs to the most undersampled systems, not only in regard to bacterial parameters.

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The Arctic Ocean is characterized by a number of features that distinguish it from other oceanic regions. It receives 10% of the global river discharge, corresponding to an annual riverine DOC import of 23 to 25 Tg C (Opsahl et al. 1999, Anderson 2002). There is evidence that global warming is increasing Arctic river runoff (Lammers et al. 2001, Peterson et al. 2002), which probably affects biological processes on the shelf as well as the exchange of matter between the Arctic watersheds and the coastal Arctic Ocean. Up to 60% of the world's soil organic matter resides in the permafrost-dominated drainage basins of Arctic rivers (Dixon et al. 1994), part of which could be mobilized by permafrost thaw and coastal erosion. This highlights the tight coupling of the Arctic Ocean with the terrestrial catchment sites and the potential impact of terrigenous dissolved organic material (DOM) on bacterial metabolism in the estuarine and coastal environment of this high-latitude system. Bacterial processing of riverine DOM and DOM produced by phytoplankton on the Arctic shelf is likely to have a strong impact on the carbon cycle and budget of the entire Arctic Ocean because shelf areas account for approximately 35% of the Arctic Ocean's surface.

To our knowledge, only 1 study in the Lena delta and the adjacent Laptev Sea has addressed bacterial activity in an Arctic estuary (Saliot et al. 1996). Based on thymidine uptake measurements, Saliot et al. (1996) reported a higher bacterial production in the Lena river and delta and lower bacterial activity in the Laptev Sea, suggesting favorable conditions (e.g. elevated temperature, supply of labile DOC) for bacterial growth in the river and the estuary. Data on bacterial activity in coastal zones of the Arctic Ocean are available for the Barents Sea (Thingstad & Martinussen 1991), Chukchi Sea (Cota et al. 1996, Steward et al. 1996, Rich et al. 1997), Resolute Passage (Pomeroy et al. 1990) and the Franz-Joseph Land archipelago (Müller-Niklas & Herndl 1996). The general picture that has evolved is that of an active and dynamic bacterial community and the potential for relatively high bacterial production rates similar in size to those in temperate oligotrophic oceans and somewhat higher than in Antarctic waters. However, bacterial leucine incorporation rates in these studies differed significantly, ranging between <10 and >200 pM h^{-1}. Since ambient water temperatures in the course of these studies were all close to 0°C, factors other than temperature contributed to this variability. Changes in the supply and composition of organic carbon due to the variablility in sea ice coverage, sea ice patchiness, and thus primary production, have been proposed to be important factors determining the spatial and temporal differences of bacterial activity in Arctic waters (Cota et al. 1996, Müller-Niklas & Herndl 1996, Rich et al. 1997). Recent studies have shown that organic carbon produced by ice algae and released upon melting of the sea ice is rapidly used by bacteria (Amon et al. 2001) and may significantly contribute to bacterial production in surface water (Wheeler et al. 1996, Gosselin et al. 1997). Although the molecular composition of DOM is a key factor to understanding the variability of bacterial production, very few studies have linked bacterial production to concentrations and uptake of easily available substrates such as dissolved free amino acids (DFAA) or dissolved free neutral sugars (DFNS), and fewer still have determined both free sugar and DFAA uptake at the same time (Kirchman 2003). In Arctic waters, only Rich et al. (1997) have linked bacterial production to concentrations of DFAA and glucose. In their study, turnover-rate constants of these substrates were high, averaging 0.23 d^{-1} in a transect from the Chukchi Sea to the North Pole. Uptake of amino acids and glucose often exceeded bacterial production, indicating the importance of these compounds for bacterial growth. Most previous studies suggested that the bacterial carbon demand in the central parts of the Arctic Ocean could be significantly higher than total primary production (Rich et al. 1997, Sherr & Sherr 2003). A possible explanation for this imbalance is the availability of allochthonous dissolved organic carbon of terrestrial origin and/or surplus primary production exported from the shelfs to the central Arctic Ocean.

We measured bacterial production, respiration, and uptake of amino acids and glucose in the Arctic rivers Ob and Yenisei and in their estuaries and the adjacent Kara Sea. The Kara Sea receives about 40% of the total annual Arctic river and DOC discharge (Telang et al. 1991). Thus, the Kara Sea is a suitable representative location for determining the role of bacteria in terrigenous and autochthonous DOM-processing on the Arctic shelf.

MATERIALS AND METHODS

Study site and sampling. Sampling and experimental work was performed within the framework of the joint Russian-German project 'Siberian River Run-off' (SIRRO) during a cruise on RV 'Akademik Boris Petrov' to the Kara Sea in August and September 2001. The sampling locations of the investigated area covered the freshwater endmembers of the 2 major rivers, Ob and Yenisei, which discharge into the Kara Sea, the central Kara Sea and the northern Kara Sea as far as 78° N (Fig. 1).The entire study area was ice-free during sampling. At the major hydrographic stations, water samples were taken from the surface (1 to 2 m), pycnocline (6 to 20 m) and close to the bottom (10 to 300 m) using a CTD-rosette sampler equipped with Niskin

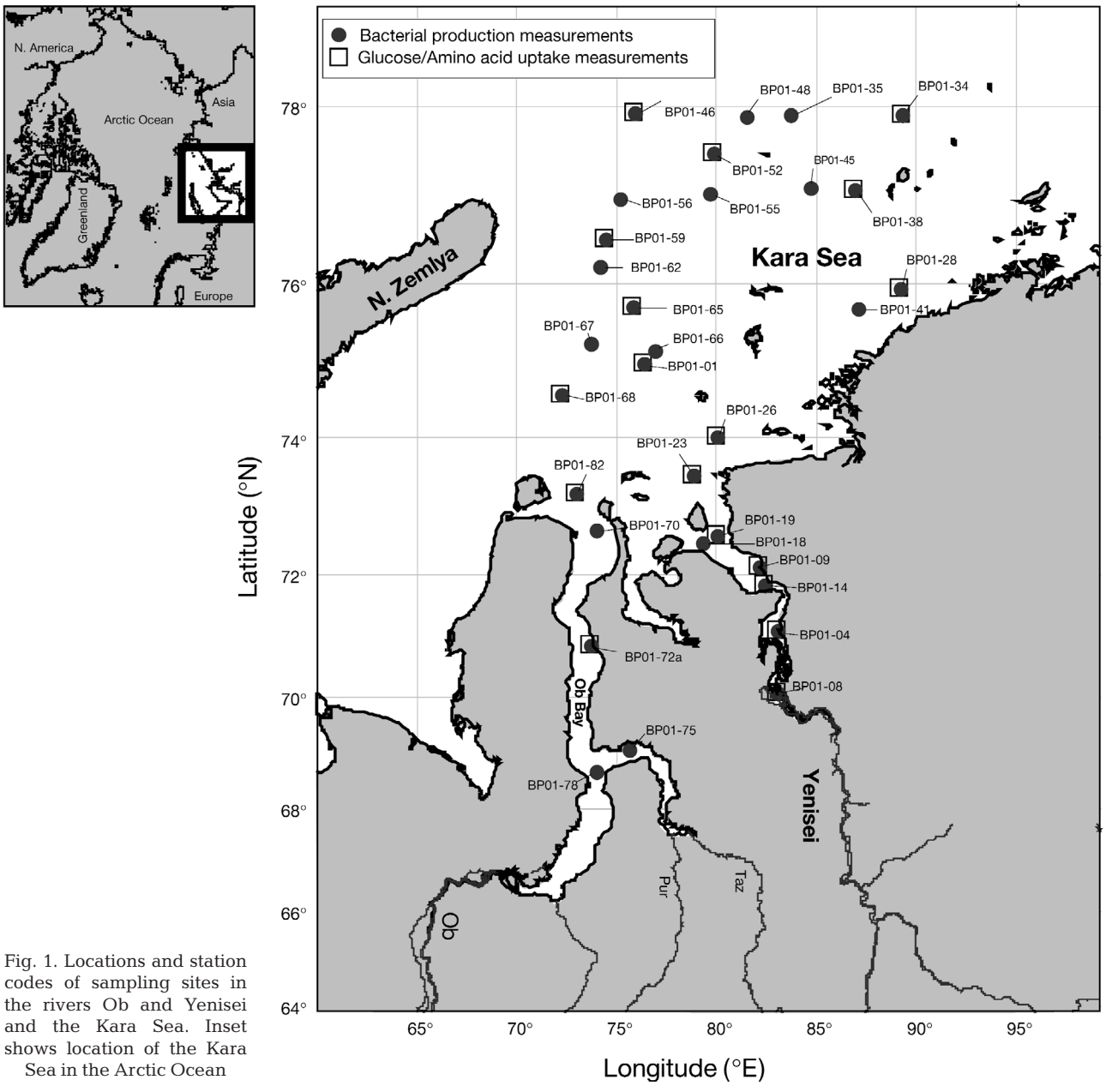

Fig. 1. Locations and station codes of sampling sites in the rivers Ob and Yenisei and the Kara Sea. Inset shows location of the Kara Sea in the Arctic Ocean

bottles. Occasionally, if larger volumes for experimental work were needed, water from the surface was collected using clean buckets or a pre-rinsed steel batomat.

Bacterial abundance and production. Samples for bacterial counts were fixed with 0.2 µm filtered 37% formaldehyde (final concentration 1.75%) and stored at 4°C in a refrigerator. We stained 5 to 10 ml of the samples with DAPI (Porter & Feig 1980), and these were filtered onto 0.2 µm black polycarbonate filters (Whatman) backed by an 0.45 µm cellulose acetate filter (Sartorius) within 2 d of sampling. Slides were stored in the freezer at −20°C. Bacterial abundance was estimated by epifluorescence microscopy with a minimum of 300 cells counted per slide in 10 to 15 different microscopic fields. Bacterial production was estimated by the leucine incorporation method in triplicate (Kirchman 1993). Tritiated leucine (specific activity 62 Ci $mmol^{-1}$; Amersham) was added to 10 ml of sample water to a final concentration of 10 nM. A control sample received 1 ml of 5% trichloroacetic acid (TCA) prior to incubation to account for unspecific sorption of tritiated leucine. After incubating in the dark for 1 to 2 h at ±2°C of the corresponding *in situ* temperature, the samples were filtered through 0.22 µm mixed cellulose ester filters (Millipore). The filters were extracted with ice-cold

5% TCA for 5 min and rinsed with filtered sample water. Incubations using water from the freshwater and marine endmembers showed that an addition of 10 nM leucine was sufficient to saturate bacterial uptake systems, and time-course experiments confirmed that uptake was linear for at least 6 h. Since *in situ* concentrations of unlabeled leucine, as determined by high-performance liquid chromatography (HPLC; see later subsection), were usually <1 nM, external dilution of the tritiated leucine was negligible. The air-dried filters were combusted in a biological material oxidizer (OX500; R. J. Harvey Instrument Corporation) and [^{3}H]-(H_2O) was trapped in scintillation cocktail (Oxysolve T, Zinsser Analytic) and radioassayed. In a previous study, combustion of filters resulted in higher recoveries of radioactivity compared to dissolution of filters in various solvents (Amon & Benner 1998). The killed control values were subtracted from the values measured in live samples. The mean coefficient of variation for radioassayed triplicates was 3.4 ± 3.7% (n = 140). Incorporation rates of leucine were converted to bacterial production assuming 1.15×10^{17} cells mol^{-1} (Kirchman 1992) and 20 fg C cell^{-1}. These factors are frequently used in the literature to estimate bacterial production and thus allow comparison with other studies. Since the above-mentioned conversion factor is derived from oceanic studies, we tested its applicability to our riverine endmembers by measuring leucine uptake and bacterial abundance over time in 0.8 µm filtered water diluted with 0.2 µm filtered water (1:9; v:v) from the Yenisei. The conversion factor thus determined (1.0×10^{17} cells mol^{-1}) was very similar to that given by Kirchman (1992).

Bacterial respiration and bacterial growth eficiency. Water from selected stations was filtered through 0.8 µm pore-size Nuclepore cartridge filters (Whatman). The removal of eukaryotes and recovery of bacterial cells (>90%) was confirmed by microscopy of filtered and unfiltered samples. The filtered water was allowed to stand for at least 30 min before it was siphoned, bubble-free, into 120 ml Winkler bottles. The samples were incubated for 36 to 48 h in the dark at *in situ* water temperature. Dissolved oxygen was measured at the start and at the end of the incubation in 4 replicate bottles with the Winkler method using a Mettler DL-21 autotitrator and potentiometric equivalence point determination (Granéli & Granéli 1991). The mean coefficient of variation of the method was 0.24 ± 0.21% (n = 33). Bacterial respiration is only reported if the means of the oxygen concentrations at the start and at the end of the incubations differed signifcantly (Student's *t*-test). Assuming a respiratory quotient of 1, bacterial growth efficiency (BGE) was calculated as BGE (%) = bacterial production/(bacterial production + bacterial respiration) × 100.

Amino acid and glucose turnover. Uptake of a [^{3}H]-amino acid mixture (Amersham; amino acid composition similar to that of algal protein) and D-[6-^{3}H]-glucose (specific activity of 30 Ci mmol^{-1}, Amersham) was measured separately. The labeled compounds were added to 10 ml of tripilicate samples (final concentration 0.5 nM). Controls were killed with 1 ml of 5% TCA prior to incubation. The samples were incubated in the dark for 4 to 12 h at ±2°C of the respective *in situ* temperatures. After incubation, the samples were filtered through 0.22 µm mixed cellulose filters (Millipore). The filters were rinsed twice with 5 ml of filtered water from the respective sampling sites and allowed to dry. After combustion of the filters (see above) the trapped [^{3}H]-(H_2O) was radioassayed. Control values were subtracted from the values measured in live samples. The turnover-rate constant was calculated as the fraction of radioactivity incorporated by the bacterial cells on the filters and the added radioactivity per unit time.

Carbon limitation of baterial growth. Carbon limitation of bacterial growth was tested at 5 stations located in the rivers Ob and Yenisei and in the Kara Sea. Unfiltered surface water samples were incubated in 2 l polycarbonate bottles in the dark at the respective surface water temperatures. Prior to incubation, 1 bottle received 2 µM of glucose as carbon source and 1 bottle with no addition served as control. Subsamples for bacterial production measurements were taken at regular time intervals over a period of 3 d.

Impact of riverine water on bacterial growth in the Kara Sea. Bacteria-free water (0.5 l, 0.2 µm filtered) from the Yenisei endmember was added to polycarbonate bottles containing 1.5 l of raw surface water of different salinity (12.9 and 28.4, respectively) from the Kara Sea. Each experiment consisted of 2 replicates and 1 control bottle. Each control bottle received 1.5 l of surface water and 0.5 l of 0.2 µm filtered water from the respective sampling sites. The bottles were incubated in the dark and subsamples for bacterial production measurements were taken over a period of 3 to 4 d.

Amino acid and carbohydrate analyses. DFAA and DFNS were determined on GF/F-filtered water samples stored at −18°C in combusted ampules. DFAA were analysed by reversed-phase HPLC with fluorimetric detection after precolumn derivatization with *o*-pthaldialdehyde (Lindroth & Mopper 1979). The protocol and the chromatographic system was the same as described by Fitznar et al. (1999), and allowed separation of D/L enantiomers using *N*-isobutyryl-D-cystein as chiral reagent for derivatization. Quantification was performed relative to an amino acid standard mixture. The mean difference between duplicate analyses was 7.7 ± 9 nM (n = 8).

DFNS were determined by anion-exchange HPLC with pulsed amperometric detection using a Dionex system, a Carbopac PA-1 column and an isocratic eluent of 21 mM NaOH. Desalting of samples followed the procedures described by Skoog & Benner (1997), using 3.5 ml of a mixed cation and anion exchange resin. Before desalting, the samples were spiked with 50 nM deoxyribose as internal standard. The mean difference between duplicate analyses of DFNS was 3.1 ± 2.6 nM (n = 12)

Chlorophyll *a*. Water samples (500 to 1000 ml) were filtered through GF/F filters (Whatman). The filters were stored frozen at –18°C until further processing. The filters were extracted in 90% acetone and chlorophyll *a* (chl *a*) concentrations were determined fluorometrically.

DOC. Samples for DOC analysis were filtered through precombusted GF/F filters and stored in sealed ampules at –20°C. DOC concentrations were measured using an MQ 1001 TOC analyzer (Qian & Mopper 1996) and high-temperature catalytic oxidation. Each sample was analyzed in duplicate with a minimum of 3 replicate injections. The coefficient of variation was <2%. The performance of the instrument was routinely tested using an international reference standard.

RESULTS

Distribution of bacterial production and respiration

In order to address general trends in the data set, we pooled stations according to surface salinities into 3 categories labeled as rivers (salinity 0 to 1), estuaries (salinity 5 to 15) and Kara Sea (salinity >20). Mean bacterial production in surface waters expressed as leucine incorporation was highest in the rivers Ob (295 pM h^{-1}) and Yenisei (197 pM h^{-1}), decreased towards stations in the estuaries (105 pM h^{-1}), and was lowest (45 pM h^{-1}) at sampling sites with a salinity above 20 in the open Kara Sea (Fig. 2). Bacterial production also decreased with increasing depth at stations in the estuaries and the open Kara Sea, where a pronounced pycnocline separated the surface layer from the bottom layer. Bacterial production close to the bottom was on average about 25% of that measured near the surface (Fig. 2). Pooled with respect to surface salinities, the bacterial production in the rivers, the estuaries and the Kara Sea averaged 13.5, 5.8 and 2.4 µg C l^{-1} d^{-1}, respectively. The variability in bacterial production was largest in the river Ob: values as high as 25 µg C l^{-1} d^{-1} were encountered at the 2 southernmost stations in the Ob Bay (Fig. 1, Table 1). However, a significantly smaller production in the range of 6 to 8 µg C l^{-1} d^{-1} was measured towards the mouth of the Ob Bay at Stns BP01-70 and BP01-72a, respectively (Table 1). Bacterial abundance both in the rivers and the estuaries was high, averaging 1.51×10^6 and 1.93×10^6 cells ml^{-1}, respectively, but dropped to numbers $< 0.5 \times 10^6$ cells ml^{-1} in surface waters of the open Kara Sea (Table 1).

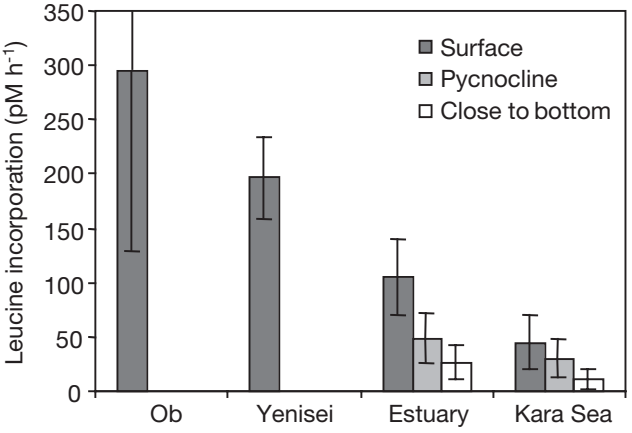

Fig. 2. Leucine incorporation in the rivers Ob (n = 4) and Yenisei (n = 6), and in the estuaries (n = 8) and the Kara Sea (n = 14). Error bars indicate ±1 SD of pooled data

Similar to bacterial production, surface phytoplankton biomass as estimated by chl *a* concentrations (Table 1) was elevated in the rivers (2.7 to 4.2 µg l^{-1}) and decreased towards the estuaries (1.1 to 2.5 µg l^{-1}) and the open Kara Sea (≤ 0.9 µg l^{-1}). Phytoplankton biomass and chl *a* concentrations correlated significantly ($r = 0.78$, $p < 0.001$), indicating a tight coupling between heterotrophic bacterial activity and primary production over the whole salinity range from the freshwater to the marine endmember (Fig. 3).Water

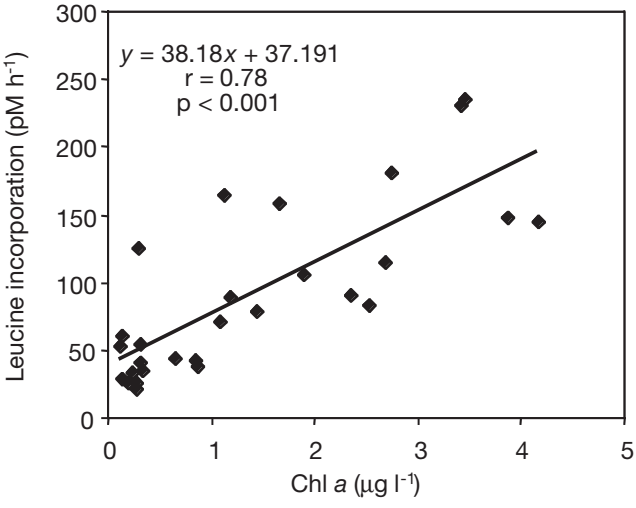

Fig. 3. Chlorophyll *a* concentrations versus leucine incorporation in surface samples from the rivers Ob and Yenisei and the Kara Sea

Table 1. Physical, chemical and bacterial parameters in surface waters (1 to 2 m depth) of rivers Ob and Yenisei, their estuaries, and in the Kara Sea. Bacterial abund.: abundance; prod. production based on conversion factor for leucine uptake of 1.15×10^{17} cells mol^{-1} (Kirchman 1992) and 20 fg C $cell^{-1}$; integr. prod.: production integrated over depth of 25 m using bacterial production data from surface, pycnocline and bottom layer at respective sampling stations; DFAA: dissolved free amino acids; Gluc: glucose; DOC: dissolved organic carbon. Station codes as in Fig. 1

Station	Latitude (°N)	Longitude (°E)	Temperature (°C)	Salinity	Chl *a* (µg l^{-1})	Bacterial			Turnover (d^{-1})		DOC (µM C)
						Abundance (10^6 ml^{-1})	Production (µg C l^{-1} d^{-1})	Integr.-prod. (mg C m^{-2} d^{-1})	DFAA	Gluc.	
Ob											
BP01-70	72°40.16′	74°00.22′	9.2	0.9	2.7	1.21	6.30	74			685
BP01-72a	70°49.88′	73°44.34′	11.5	0	4.2	1.68	7.98	154	1.89	2.18	913
BP01-75	69°05.04′	75°41.85′	10.4	0			25.40	152			
BP01-78	68°40.07′	73°59.74′	11.4	0			24.84	173			1168
Yenisei											
BP01-04	71°05.50′	83°06.20′	13.8	0	3.5	1.40	12.96	246	2.21	1.77	
BP01-05	70°45.50′	83°33.10′	13.9	0		1.61					698
BP01-07	70°21.08′	82°59.14′		0		1.28					692
BP01-08	70°04.10′	83°03.90′	14.5	0	3.9		8.13	203	1.37	1.25	
BP01-09	72°06.90′	82°10.70′	12.7	0	3.4		12.73	140			
BP01-14	71°49.30′	82°27.20′	12.8	0	2.8	1.61	10.02	210	1.99		773
Estuaries											
BP01-18	72°28.30′	79°20.40′	9.2	8.6	2.5		4.57	59			
BP01-19	72°35.70′	80°06.40′	9.6	6	1.7	1.80	8.75	91	1.92	1.63	
BP01-23	73°29.00′	78°50.90′	10.2	5.1	1.1	1.65	9.10	61	2.85	1.36	
BP01-26	74°00.00′	80°01.40′	7.9	12.4	1.2	1.98	4.90	62	1.19	0.62	
BP01-66	75°10.04′	76°55.13′	7.1	13	1.1		3.91	40			520
BP01-67	75°14.65′	73°45.78′	7.4	11.3	2.4		4.97	57			370
BP01-68	74°35.05′	72°14.97′	7.8	9.9	1.5	1.78	4.32	62	0.93	1.07	530
BP01-82	73°11.83′	73°01.65′	6.7	9.9	1.9	2.46	5.80	75	1.27	1.43	536
Kara Sea											
BP01-01	74°59.12′	76°23.41′	5.8	26.6	0.1	0.46	3.31	79	0.18	0.04	224
BP01-28	75°56.34′	89°15.90′	2.6	24.4	0.3	0.25	6.89	74	0.78	0.28	
BP01-34	77°54.29′	89°20.15′	1.4	29.1	0.2	0.23	1.44	54	0.20	0.31	138
BP01-35	77°54.31′	83°45.94′	3.2	28.8	0.2		1.81	40			160
BP01-38	77°05.29′	86°55.48′	2.7	29.2	0.1		1.54	33	0.43	0.23	148
BP01-41	75°41.40′	87°07.80′	4.0	23.8	0.3		1.14	22			188
BP01-45	77°06.83′	84°44.00′	3.3	29	0.1	0.29	2.89	69			157
BP01-46	77°55.43′	75°57.35′	4.2	26.4	0.3	0.36	1.88	45	0.53	0.33	236
BP01-48	77°53.49′	81°29.94′	3.7	26.4	0.3	0.39	2.99	53			192
BP01-52	77°29.94′	79°52.00′	4.1	25.4	0.3		2.21	33	0.39	0.66	224
BP01-56	76°59.58′	75°11.48′	5.4	21.7	0.9		2.08	31			362
BP01-59	76°31.16′	74°30.95′	5.2	21.9	0.7	0.47	2.45	26	0.59	0.48	298
BP01-62	76°12.05′	74°12.15′	4.7	21.7	0.3		1.44	12			251
BP01-65	75° 42.98′	75° 50.79′	5.9	19.1	0.9		2.34	24	0.27	0.76	365

temperature is another factor with a potential impact on bacterial production. Surface water temperatures decreased from up to 14.5°C in the rivers to <6°C in the Kara Sea (Table 1) and correlated significantly with bacterial production ($r = 0.67$, $p < 0.001$).

The measurement of bacterial respiration and bacterial production in water samples from the same sampling sites and depths allows an estimate of the BGE, an index that describes the bacterial efficiency in converting assimilated organic carbon to biomass. Bacterial respiraton rates in surface layers ranged between undetectable and 1.96 µM O_2 d^{-1}, the latter being measured in the River Ob (Table 2). In contrast to surface samples, oxygen concentrations in samples taken from pycnocline layers always differed significantly between the start and end of incubation with respiration rates ranging between 0.34 and 2.19 µM O_2 d^{-1}. Interestingly, bacterial respiration rates in the pycnocline layer were often higher than in the relevant surface sample, despite a lower bacterial production. This resulted in relatively low BGEs for pycnocline samples, ranging between 10 and 15% (Table 2). The BGE in the surface layer averaged 27% (range 12 to 39%), indicating that factors controlling the conversion of DOC to bacterial biomass (e.g. the quality of DOC) were more favorable at the surface than at depth in the Kara Sea.

Table 2. Bacterial respiration (BR), bacterial production (BP) and bacterial growth efficiencies (BGE). BGE (BGE = BP/(BP + BR) × 100; respiratory quotient = 1) in the rivers Ob, Yenisei and in the adjacent Kara Sea. Station codes as in Fig. 1. ns: no significant difference between start and end of incubation

Station	Depth layer	Bacterial		BGE
		Respiration ($\mu M\ O_2\ d^{-1}$)	Production ($\mu g\ C\ l^{-1}\ d^{-1}$)	(%)
Ob BP01-72a	Surface	1.96	7.98	25
Yenisei BP01-04	Surface	ns	12.96	
Estuary BP01-68	Surface	0.57	4.32	39
	Pycnocline	1.01	2.19	15
Kara Sea BP01-01	Surface	ns	3.31	
	Pycnocline	2.19	3.01	10
Kara Sea BP01-45	Surface	0.62	2.89	28
	Pycnocline	1.28	2.81	15
Kara Sea BP01-52	Surface	0.41	2.21	31
	Pycnocline	0.92	1.2	10
Kara Sea BP01-59	Surface	n.s.	2.45	
	Pycnocline	0.34	0.67	14
Kara Sea BP01-65	Surface	1.38	2.34	12
	Pycnocline	0.77	1.15	11

Amino acid and glucose fluxes

Concentrations of DFAAs and dissolved glucose were very low throughout the rivers, the estuaries and the Kara Sea, with average concentrations of surface, pycnocline and bottom samples of <50 nM for DFAA and <10 nM for glucose (Table 3). DFAA and glucose pools in the rivers Ob and Yenisei and their estuaries were highly dynamic, as indicated by high turnover-rate constants for surface samples in the range of 0.9 to 2.9 d^{-1} for DFAA and 0.6 to 2.2 d^{-1} for glucose (Table 1). The corresponding turnover-rate constants at stations in the Kara Sea (Table 1 & 3) were somewhat lower (DFAA 0.18 to 0.78 d^{-1}, mean 0.43 d^{-1}; glucose 0.04 to 0.76 d^{-1}, mean 0.39 d^{-1}) but still substantial. Averaged turnover-rate constants of DFAA and glucose measured in samples taken from the pycnocline layer and above the bottom of estuarine and Kara Sea stations did not differ substantially from surface samples (Table 3). DFAA and glucose turnover-rate constants correlated significantly ($r = 0.81$, $p < 0.001$). All measured turnover-rates are conservative because we did not determine respiration of the assimilated substrates. Combining DFAA and glucose concentrations with the corresponding turnover-rate constants allows an estimate of both substrate uptake and the contribution of these substrates to bacterial production at the respective sampling sites. The latter is possible because we measured net substrate uptake as well as net bacterial production with the applied methods. Uptake of DFAA usually exceeded glucose uptake several-fold (Table 3). Similar to the measured turnover-rate constants, mean uptake rates of DFAA and glucose were higher in the rivers (2.82 and 0.81 $\mu g\ C\ l^{-1}\ d^{-1}$, respectively) and the estuaries (up to 2.68 and 0.21 $\mu g\ C\ l^{-1}\ d^{-1}$, respectively) and dropped significantly at stations in the Kara Sea (0.6 and ≤0.13 $\mu g\ C\ l^{-1}\ d^{-1}$, respectively). DFAA were more important in supporting bacterial production than glucose. DFAA uptake averaged 24% of the bacterial production at 2 stations in the river Yenisei and in 2 surface samples taken in the estuaries (Table 3). The contribution of DFAA to bacterial production was slightly higher in surface (30% ± 18, n = 7) and pycnocline (37% ± 24, n = 6) samples of the open Kara Sea. The highest mean contribution of DFAA uptake to bacterial production (about 100%) and also the largest variations were encountered in the pycnocline layer of the estuaries and in samples taken above the bottom in the Kara Sea. In contrast, glucose on average supported only <10% of bacterial production at the investigated sampling sites and depth layers (Table 3).

Table 3. Turnover-rate constants, concentrations, uptake and contribution to bacterial production (BP) of dissolved free amino acids (DFAA) and glucose in the Kara Sea system. Mean ± SD; range when n = 2 (no. of samples). nd: not determined

Location	Turnover-rate constants (d^{-1})		DFAA (nM)	Glucose (nM)	Uptake as $\mu g\ C\ l^{-1}\ d^{-1}$		Uptake as % of BP	
	DFAA	Glucose			DFAA	Glucose	DFAA	Glucose
Rivers								
Ob	1.89 (n=1)	2.18 (n=1)	11 (n=1)	2 (n=1)	nd	nd	nd	nd
Yenisei	1.86±0.36 (n=3)	1.54±0.22 (n=3)	16–39 (n=2)	3–10 (n=2)	1.84–3.75 (n=2)	0.34–1.27 (n=2)	18–29 (n=2)	3–10 (n=2)
Estuaries								
Surface	1.63±0.69 (n=5)	1.22±0.35 (n=5)	12–17 (n=2)	2–3 (n=2)	0.74–1.82 (n=2)	0.2–0.22 (n=2)	17–31 (n=2)	4–5 (n=2)
Pycnocline	1.75±0.45 (n=5)	1.14±0.29 (n=5)	11–54 (n=2)	1–3 (n=2)	0.9–5.9 (n=2)	0.06–0.19 (n=2)	41–165 (n=2)	2–9 (n=2)
Above bottom	1.05±0.47 (n=5)	0.6±0.24 (n=5)	11–14 (n=2)	1 (n=2)	0.26–0.86 (n=2)	0.02–0.04 (n=2)	17–56 (n=2)	1–3 (n=2)
Open Kara Sea								
Surface	0.43±0.19 (n=8)	0.39±0.22 (n=8)	48±52 (n=7)	5±1.5 (n=7)	0.6±0.33 (n=7)	0.13±0.07 (n=7)	30±18 (n=7)	6±2.7 (n=7)
Pycnocline	0.99±0.47 (n=8)	0.52±0.23 (n=8)	19±16 (n=7)	2±1.1 (n=6)	0.56±0.26 (n=6)	0.06±0.03 (n=6)	37±24 (n=6)	5±2.6 (n=6)
Above bottom	0.77±0.45 (n=8)	0.28±0.2 (n=7)	18±14 (n=6)	3±2.1 (n=5)	0.64±0.85 (n=5)	0.07±0.08 (n=5)	104±62 (n=5)	9±5.5 (n=5)

Response of bacterial growth to glucose and river water addition

The addition of glucose significantly increased bacterial production (17 to 43%) relative to control treatments (no addition) in incubations with surface water from the rivers, the estuaries and the Kara Sea, indicating carbon limitation of bacterial growth in the rivers and throughout the Kara Sea (Fig. 4). It is noteworthy that the added glucose concentrations (2 µM final concentration) was more than 2 orders of magnitude higher than *in situ* glucose concentrations, but accounted for only <2 and <8% of ambient DOC measured in the rivers and the Kara Sea, respectively (Table 1). To assess the impact of riverine DOM on the growth of bacterial communities in the Kara Sea, we mixed 0.2 µm filtered water from the Yenisei river with unfiltered water samples of different salinities (28 and 13 psu) from the Kara Sea, and measured leucine incorporation over time (Fig. 5). In both experimental assays, leucine incorporation in the 2 replicates that received riverine DOM was similar to (Fig. 5A) or lower than (Fig. 5B) in the control treatments that received no riverine DOM. This suggests that riverine DOM was largely refractory. Bacterial production increased over time in both experiments and in all treatments; this can be explained by dilution and subsequent regrowth of the bacterial community upon addition of 0.2 µm-filtered river or sample water (see 'Materials and methods'). In fact, bacterial numbers in the different treatments approximately doubled during the course of the incubation (data not shown). At the final stage of 1 of the experiments, we added glucose (2 µM) to the different treatments in order to verify a response of the bacterial communities to the presence of labile carbon (Fig. 5A). Since leucine incorporation in all treatments significantly increased after the addition of glucose, it is conceivable that riverine DOM contained no significant amounts of labile DOM, thus causing no increase in bacterial production relative to control treatments.

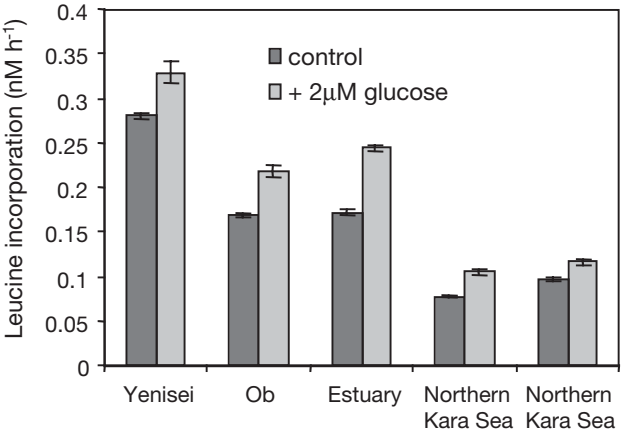

Fig. 4. Leucine incorporation upon addition of glucose to surface water samples from the rivers Ob and Yenisei and the Kara Sea. Incubations were performed at *in situ* water temperature. Samples for leucine incorporation measurements were taken 23 to 32 h after start of incubations. Error bars indicate ±1 SD of triplicate samples from relevant incubation bottle

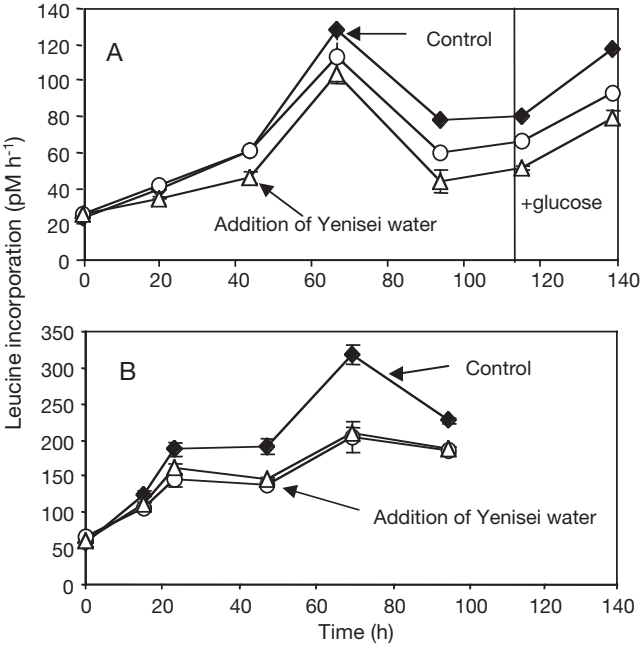

Fig. 5. Leucine incorporation upon addition of sterile-filtered Yenisei water to untreated surface water from (A) the Kara Sea (salinity 28.4) and (B) the estuary (salinity 12.9). Controls received no Yenisei water, but filtered water from relevant sampling site. Incubation experiments consisted of 1 control bottle and 2 bottles supplemented with Yenisei water. In (A) the vertical line indicates time when glucose was added to incubations to check for response to addition of labile carbon. Error bars indicate ±1 SD of triplicate samples from relevant incubation bottle

DISCUSSION

This study is the first to present data on bacterial activity and substrate fluxes in the freshwater endmembers and estuaries of the Arctic rivers Ob and Yenisei and the adjacent Kara Sea. Our data set, like most available data from the Arctic Ocean, lacks seasonal resolution because of the prevailing ice conditions that allow sampling only within a relatively short time-window. However, our 5 wk sampling period in August/September was long enough to integrate variations in biological activity during the summer/early autumn season, covering a significant portion of the hydrographs of the Ob and Yenisei and the short growing season in this high-latitude system.

Table 4. Comparison of surface leucine (leu) incorporation rates and bacterial abundances reported for surface waters of the Arctic Ocean and selected perennially cold waters in Antarctica. Sal.: salinity

Location	Season	Water temperature (°C)	Ice coverage (%)	Leu incorporation (pM h^{-1})	Bacterial abundance (cells ml^{-1})	Source
Arctic Ocean						
Arctic shelf	Jun 88	<0	100	0.0026[a]	5×10^5	Pomeroy et al. (1990)
Resolute Passage	Jun 88	<0	100	0.3[a]	2.4×10^5	Pomeroy et al. (1990)
Franz-Joseph Land Archipelago	Jul 95	<0	50–100	21–178	$2.1–5.8 \times 10^5$	Müller-Niklas & Herndl (1996)
Central Arctic	Jul–Aug 94	<0	80–100	12–70	$0.5–1.4 \times 10^6$	Rich et al. (1997)
Central Arctic	Oct 97–Sep 98	<0	100	<0.1–4.9		Sherr & Sherr (2003)
Bering Sea/Chukchi Sea	Aug–Sep 92			24–228	$2.1 \times 10^5–2.1 \times 10^6$	Steward et al. (1996)
Chukchi Sea	Aug 93	<0	>50	1–8	$2–5 \times 10^5$	Cota et al. (1996)
Chukchi Sea	Jul–Aug 94	<0	50–70	90–200	$4–6 \times 10^5$	Rich et al. (1997)
Kara Sea						
Ob River (Sal. 0)	Sep 01	9–12	0	114–471	$1.2–1.7 \times 10^6$	This study
Yenisei River (Sal. 0)	Aug 01	13–16	0	147–235	$1.4–1.6 \times 10^6$	This study
Kara Sea Estuary (Sal. 5–15)	Aug–Sep 01	6–10	0	71–165	$1.6–2.4 \times 10^6$	This study
Open Kara Sea (Sal. >20)	Aug–Sep 01	1–6	0	21–125	$2–5 \times 10^5$	This study
Antarctica						
Ross Sea	Nov–Dec 97	<0	50–100	3.6 ± 4.5[b]		Ducklow et al. (2001)
Ross Sea	Jan–Feb 97	<0	0	44 ± 22[b]		Ducklow et al. (2001)
Weddell-Scotia Sea	Jan–Feb 98	−1.4–7.8	0	0.25–4.6	$1.3 \times 10^5–1.1 \times 10^6$	Morán et al. (2001)

[a]Added leucine (0.7 nM) probably too low to saturate uptake; [b]average ±1 SD of samples in upper 50 m

Bacterial activity in the Kara Sea system

Based on the limited available information from coastal environments of the Arctic Ocean, there appears to be some variation but no major differences among the various coastal seas with respect to bacterial production despite significant differences in riverine discharge and sea ice coverage in the respective areas during the sampling periods (Table 4). Bacterial production in surface waters of the Kara Sea, as indicated by the leucine incorporation rates (21 to 125 pM h^{-1}), was in the same range as reported in most of the few other studies that performed measurements in coastal seas of the Arctic Ocean, e.g. the Chukchi Sea (90 to 200 pM h^{-1}; Rich et al. 1997), Bering Sea/Chukchi Sea (24 to 228 pM h^{-1}; Steward et al. 1996) or in the vicinity of the Franz-Joseph Land Archipelago (21 to 178 pM h^{-1}; Müller-Niklas & Herndl 1996). Bacterial abundance was also similar in these studies, generally ranging between 2 and 6×10^5 cells ml^{-1}. However, Cota et al. (1996) reported significantly lower uptake rates (1 to 8 pM h^{-1}) in the Chukchi Sea. Indications for substantial interannual variations in bacterial production have been found in the central Artcic Ocean, where Rich et al. (1997) measured leucine incorporation rates between 12 and 70 pM h^{-1}, whereas in a recent study by Sherr & Sherr (2003) leucine incorporation was much smaller (<0.1 to 4.9 pM h^{-1}). It is noteworthy that no ice coverage was present during our late–summer sampling period and water temperatures were slightly elevated. In contrast, most other studies in the Arctic Ocean occurred earlier in the year, with up to 100% ice coverage and water temperatures below 0°C. Compared to perennially cold waters close to the Antarctic continent, coastal arctic environments appear to have the potential to support a similar or even higher bacterial production in surface waters (Table 4).

One of our major findings is that mean bacterial production in surface waters of the rivers Ob and Yenisei (mean 13.5 µg C l^{-1} h^{-1}, range 6.3 to 25.4 µg C l^{-1} h^{-1}) was about 2-fold higher than in the estuarine transition zone (salinity 5 to 15) and about 5-fold higher than in the open Kara Sea (salinity >20), indicating an inverse relation between bacterial activity and salinity. The high bacterial activity in the freshwater endmembers of the Ob and Yenisei is corroborated by high turnover-rate constants of DFAA (1.89 to 2.21 d^{-1}) and glucose (1.25 to 2.18 d^{-1}), and high bacterial abundance (1.21 to 1.68×10^6 cells ml^{-1}). The only other study that has addressed bacterial production along the salinity gradient in a coastal arctic environment is that of Saliot et al. (1996). Similar to our results, Saliot et al. (1996) reported a higher bacterial production (based on thymidine uptake) in the delta of the Lena river and decreasing production in the adjacent Laptev Sea. Studies in other river–estuary systems found highest bacterial production values in the freshwater endmembers (Hoch & Kirchman 1993, Troussellier et al. 2002) or at higher salinity ranges (Chin-Leo & Benner 1992).

Chl *a* concentrations were significantly correlated with bacterial production data in the surface layer of

the rivers and the Kara Sea ($p < 0.001$, $r = 0.78$), and explained about 60% of the variance of bacterial activity in the Kara Sea system, indicating a tight temporal and spatial coupling between phytoplankton biomass (and probably primary production) and bacterial growth. Consequently, the turnover-rate constants of DFAA and glucose, labile substrates that are directly (e.g. cell lysis) or indirectly (e.g. grazing) released by phytoplankton cells, were also significantly correlated with bacterial production ($p < 0.001$, $r = 0.75$ and $p < 0.001$, $r = 0.61$, respectively). Coupling of primary production and bacterial production is a common feature in a variety of aquatic systems (Cole et al. 1988) and has been described for the Chukchi Sea and the central Arctic Ocean (Rich et al. 1997).

In addition to the supply of fresh phytoplankton-derived DOM, the elevated water temperature in the Ob and Yenisei could have favored bacterial activity in the freshwater endmembers relative to the estuaries and the open Kara Sea. The average surface water temperature of stations in the rivers was 12.2°C; this decreased by 4°C degrees towards estuarine stations and was lowest (4.2°C) at stations in the open Kara Sea. Temperature covaried with bacterial production ($p < 0.001$, $r = 0.67$), explaining 45% of the variability in bacterial growth. The effect of temperature on bacterial growth is debated in the literature (Pomeroy et al. 1991, Rivkin et al. 1996, Pomeroy & Wiebe 2001). During the course of a 3 yr study in the Delaware Estuary (Hoch & Kirchman 1993), temperature was the most important factor in predicting bacterial production, and a dependency of bacterial growth rates on temperature was evident when the water temperature fell below 12°C. In the Chesapeake Bay, temperature explained 40% of the variabiltiy in bacterial production during all seasons (Ducklow & Shia 1993). Although water temperature appears to have a profound effect on bacterial activity, bacterial production rates in cold waters can be high, as demonstrated for the Barents Sea (Thingstad & Martinussen 1991). Based on the data of 66 published studies, Rivkin et al. (1996) could not find significantly different specific growth rates for bacteria from warm (≥4°C) and cold (≤4°C) waters, indicating that bacteria in cold waters are well adapted to lower temperatures. The same is true for this study, when we compare the mean bacterial growth rate (production divided by bacterial biomass) in surface waters of the rivers Ob and Yenisei (0.31 d^{-1}) with that calculated for the open Kara Sea (0.49 d^{-1}). It is also evident that temperature does not explain the variation in bacterial production in the river Ob, where 3- to 4-fold higher values were measured in the southern part of the Ob Gulf than at stations located closer to the mouth of the river, without corresponding changes in water temperature among stations. Furthermore, the addition of labile carbon to water samples of differing temperature from the rivers and the Kara Sea resulted in an overall increase in bacterial production (Fig. 4), indicating that the availability of carbon, and not temperature, was a crucial factor in determining bacterial growth.

Concentrations and fluxes of free sugars and amino acids

Substrate availability appears to be a key factor in governing bacterial growth in the Kara Sea system, rendering the measurement of fluxes of important carbon sources such as DFAA and DFNS (glucose) a prerequisite to addressing and understanding variations in bacterial activity. The mean DFAA and glucose (the only measurable DFNS) concentrations measured in our study were generally very low (<50 and <10 nM, respectively) and showed no consistent pattern across the rivers towards the Kara Sea (Table 3). The measured concentrations that were often close to the detection limit of the HPLC systems probably reflect the lower threshold limits of bacterial transport systems, indicating both a high demand for these labile substrates and the presence of efficient uptake mechanisms in the bacterial communities of this high-latitude system. Our findings are similar to results from studies in temperate waters where low DFAA concentrations (<50 nM) were measured in combination with high turnover-rates and a tight coupling of DFAA to bacterial production (e.g. Fuhrman 1987, 1990). In contrast to our study, Rich et al. (1997) found relatively high concentrations of DFNS in the range of 50 to 100 nM, and about 3 to 4 times higher DFAA concentrations (>200 nM) in surface waters of the Chukchi Sea and towards the North Pole. Since the corresponding turnover-rate constants in their study were similar to those we measured in the Kara Sea, it is unlikely that a less active bacterial community caused the 'accumulation' of labile substrates in the Chukchi Sea. In fact, the bacterial leucine incorporation in the Chuckchi Sea (90 to 200 pM h^{-1}) was even slightly higher than in the Kara Sea (21 to 125 pM h^{-1}). The release of dissolved amino acids and sugars from melting sea ice causing a transient increase in concentrations could be the reason for the discrepancy (Pomeroy et al. 1990, Amon et al. 2001). For example, the concentrations of total (mono- and polymers) neutral sugars and total amino acids in an ice floe were reported to be as high as 2620 and 1831 nM, respectively, together contributing 21% to the DOC in the sea ice (Amon et al. 2001). These numbers are considerably higher than ambient concentrations in Arctic Ocean surface water. Only a few other studies measured concentrations of free amino

acids and glucose in the Arctic Ocean. Cota et al. (1996) found DFAA concentrations in the Chukchi Sea to range between 30 and 200 nM. Pomeroy et al. (1990) found concentrations of individual DFAA to be mostly <20 nM, which was the detection limit of their system. Similar to our results, riverine DFAA concentrations were low (50 to 70 nM) in the Lena Delta (Lara et al. 1998). Low DFNS concentrations (based on HPLC measurements using pulsed amperometric detection) are a typical feature in a variety of marine regions, with glucose being the dominant and often only detectable sugar. Glucose concentrations in water samples from the Equatorial Pacific (August to September; Rich et al. 1996), North Pacific (Skoog et al. 2002), Gulf of Mexico (Skoog et al. 1999), Ross Sea and Antarctic Polar Front Zone (Kirchman et al. 2001) or in the Delaware Estuary (Kirchman & Borch 2003) were generally below 15 nM, suggesting that glucose concentrations of >50 nM, as reported for the Arctic (Rich et al. 1997) and the Equatorial Pacific (February–March; Rich et al. 1996) are rather the exception than the rule.

Unlike the homogeneously low glucose and DFAA concentrations, flux parameters like turnover-rate constants and uptake of glucose and DFAA are more instructive in explaining the higher bacterial activity in the rivers and the estuaries of the Kara Sea system. Mean turnover-rate constants and uptake rates of glucose and DFAA were about 2- to 6-fold higher in surface waters of the freshwater endmembers and the estuaries compared to the open Kara Sea (Table 3). Together with low concentrations of these substrates, this infers an increased supply of free sugars and amino acids in the riverine and estuarine zones. This scenario seems likely, since phytoplankton biomass, the ultimate source of dissolved labile substrates, was significantly higher in these zones (1.1 to 4.2 µg chl *a* l^{-1}; Table 1) than at the corresponding sampling stations in the Kara Sea (0.1 to 0.9 µg chl *a* l^{-1}). Although lower than the numbers in the rivers and their estuaries, the mean turnover-rate constants of glucose and DFAA in surface waters of the Kara Sea were still relatively high (0.39 and 0.43 d^{-1}, respectively), indicating rapid uptake of labile DOM components in this coastal Arctic environment. High turnover-rate constants of both glucose and DFAA (average: 0.23 d^{-1}) have also been reported across a transect from the Chukchi Sea towards the Northpole (Rich et al. 1997). These numbers are more than 1 order of magnitude higher than previous reports of the turnover of individual amino acids in the Chukchi Sea (Cota et al. 1996), Resolute Passage and the Arctic Ocean (Pomeroy et al. 1990). Rate constants for free glucose were significantly lower (<0.08 d^{-1}) in surface waters of the Ross Sea and the Polar Front Zone (Kirchman et al. 2001) whereas data of glucose turnover covered a wide range (0.02 to 0.6 d^{-1}) in a number of other oceanic regimes, e.g. the Equatorial Pacific (Rich et al. 1996), Gulf of Mexico (Skoog et al. 1999) and the North Pacific (Skoog et al. 2002).

On average, DFAA and free glucose uptake together supported between 28 and 37% of bacterial production in surface waters of the sampling area (Table 3), indicating the substantial contribution of these substrates to bacterial growth and flux of labile DOM in this high-latitude system. The only other study that allows an intra-Arctic comparison of our data is that of Rich et al. (1997), who reported that glucose or DFAA uptake alone often exceeded bacterial production in the Chukchi Sea and the central Arctic. This substantially higher uptake to production ratio cannot be explained by the application of different factors for the conversion of leucine incorporation to bacterial production, since these were the same. Apart from the fact that ratios >1 are difficult to explain, our data may be more realistic because, in addition to DFAA and glucose, dissolved proteins and polysaccharides play an important part in sustaining bacterial growth (e.g. Keil & Kirchman 1999, Meon & Jüttner 1999, Weiss & Simon 1999) and thus may account for the remaining fraction in our study that is not covered by free substrates. It is noteworthy that in our study uptake of DFAA contributed consistently more (>4-fold) to bacterial production than glucose (and likely other free sugars), a pattern also observed in the Chukchi Sea and the central Arctic Ocean (Rich et al. 1997). This pattern may also be typical for other oceanic regions and estuaries, e.g. the Ross Sea and the Antarctic Polar Front Zone (Kirchman et al. 2001), Gulf of Mexico (Skoog et al. 1999), the North Pacific (Skoog et al. 2002) and the Delaware Estuary (Kirchman & Borch 2003) where, similar to our results, glucose generally supported less than 10% of bacterial production. A recent study (Skoog et al. 2002) indicated that uptake of glucose can be limited by low concentrations of inorganic nutrients and amino acids. In fact, particularly concentrations of ammonium and nitrate were generally very low in our surface samples (<0.5 µM of inorganic N; data not shown). However very low glucose concentrations (<10 nM) and relatively high turnover-rate constants favor the supply of glucose as the controlling factor of glucose flux in the Kara Sea system.

Bacterial utilization of terrigenous carbon in the Kara Sea

Several lines of direct and indirect evidence indicate that the large amount of terrestrially derived DOM transported by the rivers Ob and Yenisei to the Kara Sea (about 8 to 9 Tg C a^{-1}; Köhler et al. 2003) is refrac-

tory to bacterial utilization, thus playing a minor role in supplementing bacterial growth in this coastal sea. Addition of filter-sterilized water from the river Yenisei to medium and highly saline surface water of the estuaries and the Kara Sea did not stimulate bacterial production relative to the controls in short-term experiments (Fig. 5) suggesting that riverine DOM contains no higher concentration of labile compounds than that already present in waters across the salinity gradient, despite the several-fold higher DOC concentrations encountered in the freshwater endmembers (up to 1170 µM C) compared to the northern Kara Sea (150 µM C). The low riverine concentrations of labile substrates such as DFNS and DFAA confirm this assumption. In a similar study, riverine-derived substrates from the Mississippi plume enhanced heterotrophic activity in the Gulf of Mexico (Chin-Leo & Benner 1992). In our study, limitation of bacterial growth by inorganic nutrients can be excluded, since addition of labile carbon (glucose) significantly increased leucine incorporation across the salinity gradient (Fig. 4) and in 1 of our mixing experiments (Fig. 5A), indicating carbon limitation of bacterial communities both in the rivers and across the Kara Sea shelf. Since the residence time of terrigenous DOM in the Kara Sea is estimated to range between 2 and 5 yr (Schlosser et al. 1994), it is possible that semilabile components in the riverine DOC pool support bacterial growth on longer time scales. However, long-term incubation of Yenisei water (21 mo) decreased DOC concentrations only by about 30 µM (4.5%) or 0.05 µM C d^{-1} (Köhler et al. 2003), a rate too small to substantially contribute to the mean bacterial carbon demand in surface waters of the rivers (4.2 µM C d^{-1}), the estuaries (1.9 µM C d^{-1}) or the Kara Sea (0.75 µM C d^{-1}) (Table 5). One factor not considered in our dark incubations that may enhance the bioavailability of terrestrially derived compounds is photooxidation. In fact, deck incubations during our cruise revealed the photochemical reactivity of riverine DOM (Amon & Meon in press), with oxygen consumption rates ranging between 0.058 and 0.149 µM O_2 h^{-1}. However, photochemical oxygen consumption and DOC losses were about equimolar, indicating that most of the oxygen consumed was used for the formation of CO and CO_2. Furthermore, these estimates are only valid for the uppermost layer of the water column, thus representing an upper limit for photooxidation with a significant decrease in rates towards deeper layers. In summary, the conclusion was that photochemical processes are likely to be of minor importance for DOM turnover in the Kara Sea due to the prevailing physical conditions, i.e. the strong light attenuation in the water column, low solar angle, prevailing cloud conditions, and extended periods of ice cover. The recalcitrant nature of riverine DOM is corroborated by conservative mixing of terrestrial DOC and dissolved organic nitrogen along the salinity gradient of the Kara Sea (Köhler et al. 2003). Conservative behavior of riverine DOM has also been observed in the Laptev Sea, another major coastal Arctic sea receiving substantial amounts of terrestrial organic carbon through the river Lena (Cauwet & Sidorov 1996, Kattner et al. 1999). Consistent with the recalcitrant nature of Arctic riverine DOM are high concentrations of terrestrially derived DOM in surface waters of the central Arctic Ocean (Opsahl et al. 1999), and the export of terrigenous DOM from the Arctic Ocean through the Fram Strait, which is estimated to represent 12 to 50% of the annual Arctic river discharge of DOC to the Arctic Ocean. This indicates that a significant fraction of riverine DOM survives the multi-year passage through the Arctic Ocean (Opsahl et al. 1999, Amon et al. 2003).

Role of bacteria in carbon budget of the Kara Sea

Since terrestrially derived riverine DOM in the Kara Sea system appears to be of minor importance for heterotrophic bacterial growth, autochthonous production must be the main source for bacterial carbon demand (BCD). Estimates of BCD in surface waters range from 9 µg C l^{-1} d^{-1} in the open Kara Sea to 50 µg C l^{-1} d^{-1} in the freshwater endmembers (Table 5). These esti-

Table 5. Bacterial production (BP), bacterial carbon demand (BCD) and primary production (PP) (mean ± SD) in the Ob and Yenisei rivers and in the Kara Sea for surface waters and intergrated over depth. Areal data integrated over depth of 25 m using bacterial production data from surface, pycnocline and bottom layer; PP numbers integrated over euphotic depth. BP based on conversion factor for leucine uptake of 1.15×10^{17} cells mol^{-1} (Kirchman 1992) and 20 fg C $cell^{-1}$; BCD based on bacterial growth efficiency of 0.27; PP data from Vedernikov et al. (1995), with stations pooled according to their surface salinities (Sal.)

Location	Surface (µg C l^{-1} d^{-1})			Areal (mg C m^2 d^{-1})		
	BP	BCD	PP	BP	BCD	PP
Rivers (Sal. 0)	13.5 ± 7.0 (n = 8)	49.9 ± 26.1 (n = 8)	58.4 ± 33 (n = 6)	183 ± 36	676 ± 133	155 ± 114
Estuary (Sal. 5–15)	5.8 ± 1.9 (n = 7)	22.8 ±9.7 (n = 7)	44.9 ± 29.3 (n = 11)	64.4 ± 13.3	239 ± 49.3	127 ± 100
Kara Sea (Sal. >20)	2.5 ± 1.5 (n = 13)	9.2 ± 5.3 (n = 13)	8.8 ± 8 (n = 15)	43.9 ± 20.1	163 ± 74.4	57 ± 29

mates are conservative based on a conversion factor of 2.3 kg C mol^{-1} incorporated leucine (Kirchman 1992) and a bacterial growth efficiency (BGE) of 27%. In comparison, the other commonly used conversion factor of 3.1 kg C mol^{-1} (Simon & Azam 1989) would result in a higher BCD. The assumed BGE is the average of our own bacterial respiration measurement in surface waters of the rivers, estuaries and the Kara Sea (Table 3) and corresponds to the mean BGE for various coastal systems (del Gorgio & Cole 2000).

Unfortunately, we do not have parallel primary production measurements to directly compare BCD and primary production during the cruise. To our knowledge, the only comprehensive data set on primary production in the Ob and Yenisei rivers and the adjacent Kara Sea was published by Vedernikov et al. (1995) and derives from the year 1993. Similar to our study, Vedernikov et al. (1995) data cover the late summer/early fall period. Pooled according to surface salinities, their measured mean primary production (including DOC release) in surface waters (Table 5) ranged between 9 µg (open Kara Sea) and 58 µg C l^{-1} d^{-1} (rivers). These rates are close to our estimates of BCD, taking into consideration possible interannual variations between the studies in factors governing photosynthetic production and BCD. However, mean rates of areal primary production in the euphotic zone are about 2- to 4-fold lower than BCD. Discrepancies become evident based on our estimates of BCD for the entire Kara Sea water body during the sampling period. Taking the area and the volume of the Kara Sea (883 000 km^2 and 98 000 km^3, respectively) and assuming a BCD of 9.2 µg C l^{-1} d^{-1} (average of BCD in surface waters) for the upper 10 m of the water column and 2.2 µg C l^{-1} d^{-1} (average of BCD in the bottom layer) for depths >10 m, we calculate a total BCD of 0.277 Tg C d^{-1}. Again, this estimate is conservative since the volume below 10 m represents by far the larger fraction (90%) of the total volume, and BGE in deeper (and colder) water is likely to be less than 27% (thus leading to a higher BCD), as indicated by BGE in the range of 10 to 15% measured in the pycnocline (Table 2). Furthermore, the BCD in the surface waters of the estuaries (5 to 15 psu) is about twice as high as the corresponding value for the open Kara Sea, which we assigned to the upper 10 m of the water column. Even based on this conservative estimate, an annual primary production of 20 Tg C in the Kara Sea (Vinogradov et al. 2000) would only be sufficient to sustain bacterial growth for less than 2.5 mo. The carbon demand of benthic and pelagic organisms other than heterotrophic bacteria would add to the imbalance. Our estimate of the annual BCD of the Kara Sea is 44 Tg C based on a winter period of 215 d and a BCD of 0.12 µg C l^{-1} d^{-1} as measured by Sherr & Sherr (2003) during winter in the central Arctic. Thus, the more recent estimate of 37 Tg C (range 28 to 46 Tg C) of annual primary production in the Kara Sea (Sakshaug 2003), extrapolated from data of neighboring seas, may be more accurate. External sources that may level the deficit between primary production and heterotrophic carbon demand in the Kara Sea are surplus production introduced from the Barents Sea and carbon introduced to the system by coastal erosion. According to Fransson et al. (2001), the Barents Sea exports about 9.6 Tg C a^{-1} of recently produced DOC and at least part of this labile DOC is transported in water masses that pass the Northern Kara Sea close to the St. Anna Trough. In addition, recent modeling results (Karcher et al. 2003) indicate that there is a significant inflow (0.2 to 0.6 Sverdrup) of Barents Sea water to the Kara Sea via the Kara Strait, a narrow channel between Novaya Zemlya (the western boundary of the Kara Sea) and the Eurasian continent. In terms of volume flow this is about 6 to 18 times more than the river discharge of the Ob and Yenisei together. Coastal erosion of the Kara Sea coast contributes approximately 1 Tg C yr^{-1} to the system (Rachold et al. 2003). Regardless of the lack of consistent primary production estimates, our conservative BCD data indicate that primary production may be completely consumed by heterotrophic processes in the Kara Sea system with little surplus production remaining for export from the shelf to the central Arctic Ocean. From these rough estimates it becomes apparent that our current understanding of biological processes in this area is inadequate to construct a realistic carbon budget. We need better temporal resolution for primary production, especially during the spring/summer period.

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