The utilization of polysaccharides by heterotrophic bacterioplankton in the Bay of Biscay (North Atlantic Ocean)

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This study investigates the turnover of polysaccharides by heterotrophic bacterioplankton in the northern Bay of Biscay, a productive marine system on the continental margin of the temperate Atlantic Ocean. Bacterial biomass production (BBP) near the surface ranged from 0.5 to 25.7 nmol C L^{-1} h^{-1} during small phytoplankton blooms in May and June that occurred after the main spring bloom. A direct relationship between BBP and total polysaccharides strongly suggests the dependence of bacterial growth on the availability of semi-labile organic matter. Concentrations of combined glucose as well as rate constants of extracellular glucosidase activity and glucose uptake were determined to estimate the actual carbon fluxes from bacterial polysaccharide turnover. Results reveal that the degradation of polysaccharides in the upper 100 m of the water column sustained a glucose flux of 15.2–32.3 mg C m^{-2} d^{-1} that was available for bacterial consumption. The mean turnover time for polysaccharides was 170 and 165 days for α- and β-glycosidic linked polymers, respectively. Incorporation of free glucose supported 0.4–19.6% of BBP. The availability of nitrate plus nitrite (NO_x) was identified as one factor increasing bacterial incorporation of glucose in most samples. Our results demonstrate that the bacterial recycling of polysaccharides generated a significant flux of organic carbon in microbial food-webs and biogeochemical processes.

KEYWORDS: marine bacterioplankton; polysaccharides; extracellular enzymes; bacterial biomass production; organic matter degradation

INTRODUCTION

Reactive organic carbon in the ocean is enriched in polysaccharides, the primary product of photosynthesis (Cowie and Hedges, 1984; Wakeham *et al.*, 1997; Benner, 2002). Recent studies that have investigated the molecular composition and size distribution of sugars in seawater with highly sensitive analytical methods have identified glucose as the dominant neutral aldose in a variety of marine systems (Kirchman *et al.*, 2001; Sempéré, *et al.*, 2008; Kaiser and Benner, 2009; Engel *et al.*, 2011). Furthermore, these studies have shown that the majority of sugar equivalents in seawater is included

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in combined carbohydrates, while the concentration of monosaccharides is below or close to the detection limit and does not exceed 1–2% of sugars in polysaccharides. Accordingly, it must be assumed that the amount of carbon in polysaccharides is usually ~50 to 100 times higher than that in free monosaccharides. Nevertheless, little is known about the relevance of polysaccharides for production and growth of heterotrophic bacterioplankton in the ocean. While free monosaccharides are metabolized by heterotrophic bacteria on time-scales of hours to days, polysaccharides are referred to as semi-labile, which is defined by turnover times of several weeks to months (Borch and Kirchman, 1997; Skoog and Benner, 1997; Kirchman *et al.*, 2001). Semi-labile organic matter is degraded by heterotrophic bacterioplankton, when the input of labile compounds is not sufficient to meet the bacterial carbon and energy requirements. A recent modelling study suggests that semi-labile dissolved organic matter (DOM) may support 17–40% of the carbon supply to heterotrophic bacterioplankton (Luo *et al.*, 2010). On the other hand, the turnover of semi-labile compounds on time-scales of months facilitates accumulation of dissolved organic carbon (DOC) in surface waters that can be exported to the deeper ocean by convective mixing (Carlson *et al.*, 1994; Williams, 1995). Hence, polysaccharides are relevant for both bacterial carbon acquisition and carbon export.

High-molecular weight marine organic matter (>1 kDa) is of higher biological reactivity than smaller size classes (Amon and Benner, 1996). Polymers like polysaccharides are not directly accessible for marine bacterioplankton, because bacterial cells cannot take up molecules larger than 600–800 Da (Benz and Bauer, 1988; Weiss *et al.*, 1991). Therefore, bacterial polysaccharide turnover is controlled by two steps, the extracellular enzymatic cleavage of polymers and the subsequent uptake of hydrolysates. To date, in particular rates of extracellular enzymatic hydrolysis are only rarely included in marine studies quantifying polysaccharide turnover. Among extracellular enzymes involved in polysaccharide breakdown, glucosidases are known to be ubiquitously present in seawater (Christian and Karl, 1995). The activity of α- and β-glucosidases hydrolyzes α- and β-glycosidic bonds, respectively, in the terminal position of polysaccharides. Hence, these enzymatic reactions release monosaccharides that can be consumed by bacterial cells to fuel respiration or biomass production. The efficiency of extracellular enzymatic reactions, the energy demand for enzyme production and the genetic capability for synthesis of suitable extracellular enzymes are complex factors that regulate bacterial polysaccharide breakdown (Martinez *et al.*, 1996; Arrieta and Herndl, 2002). Studies on the utilization of free glucose, the product of glucosidase activity, show that glucose uptake can support a highly variable fraction of bacterial production in temperate and polar marine systems, profoundly affected by the availability of nitrogen (Rich *et al.*, 1996; Skoog *et al.*, 1999, 2002; Kirchman *et al.*, 2001).

The present study was conducted in the northern Bay of Biscay, a productive temperate shelf ecosystem in the eastern North Atlantic. The Bay of Biscay often exhibits a net heterotrophic carbon balance in summer (Serret *et al.*, 1999). Despite high primary production, community respiration can exceed rates of photosynthetic carbon fixation. Bacterial respiration contributes on average 70% to community respiration in this area, and has been shown to respond to changes in bulk organic carbon concentrations (González *et al.*, 2003). Organic carbon is mainly derived from phytoplankton blooms that provide a pulsed input of labile and semi-labile compounds to the microbial food-web. This study investigates the relevance of polysaccharide turnover for growth of heterotrophic bacterioplankton and the recycling of organic matter in the northern Bay of Biscay during small bloom events that occurred after the main spring bloom. Our study includes data on the enzymatic hydrolysis of polysaccharides by extracellular glucosidase activity as well as on the subsequent uptake and metabolization of sugars. A conceptual approach that includes rates of extracellular enzymes was applied to estimate the carbon supply provided to bacterioplankton by polysaccharide hydrolysis.

METHOD

Field sampling

Field studies were accomplished during June 2006 and May 2007 in the northern Bay of Biscay. Here, high-nutrient input is provided to the photic zone by upwelling events that sustain high productivity during spring and summer (Botas *et al.*, 1990; Wollast and Chou, 1998). Data were collected at stations along the shelf break between 47° 07′ 83″ N, 6° 92′ 01″ E and 51° 34′ 42″ N, 10° 49′ 95″ E (Fig. 1). A CTD system equipped with a rosette sampler was used to take seawater samples between 3- and 100-m depth. Bacterial biomass production (BBP) and concentrations of organic carbon, polysaccharides, chlorophyll *a*, nitrite and nitrate were measured in 2006 and 2007. In 2007 also extracellular enzyme activities and uptake rates of glucose and amino acids were determined. Figure 1 includes stations sampled in 2007 that are presented

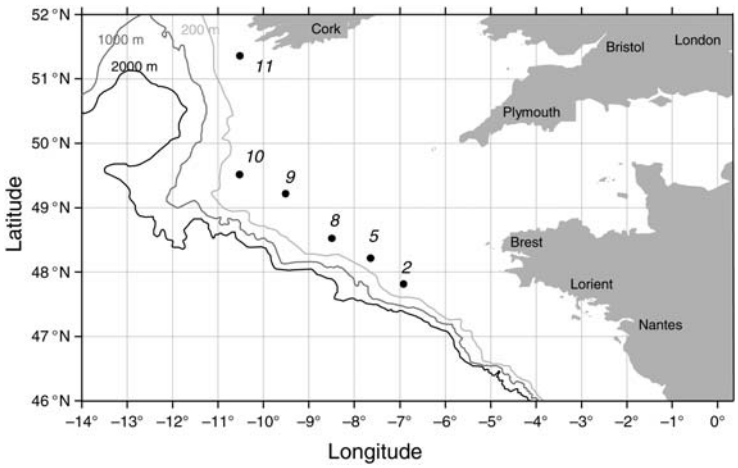

Fig. 1. Map of the study area in the northern Bay of Biscay with stations sampled during May 2007.

individually. Station 5 and station 8 were revisited 10 and 8 days later, respectively. These two profiles could not be sampled for all parameters but available data were included in data analysis and presentation.

Chemical analysis of organic carbon, polysaccharides, chlorophyll *a* and nitrate

The concentration of total organic carbon (TOC) was determined as the sum concentration of particulate organic carbon (POC) and DOC. For the analysis of POC 250 mL of sample were filtered onto combusted GF/F filters that were stored at −20°C. Prior to analysis, inorganic carbon was removed by overnight exposure to strong acid fumes. The POC content of dried filters was measured with an elemental analyzer (Fisons NA-1500). Samples for DOC were collected in combusted glass ampoules after filtration through combusted GF/F filters. Samples of 20 mL seawater were acidified with 100 μL of 85% phosphoric acid and stored at 4°C in the dark until analysis. Samples were analysed using the high-temperature combustion method (TOC -V_{CSH}, Shimadzu) (Quian and Mopper, 1996).

The analysis of polysaccharides was conducted by high-performance anion exchange chromatography (HPAEC) coupled with pulsed amperometric detection (PAD) on a Dionex ICS 3000. In this study, polymers >1 kDa were analysed because this size fraction requires extracellular enzymatic cleavage prior to bacterial uptake. Polysaccharides >1 kDa were separated from polysaccharides <1 kDa, as well as from oligosaccharides and monosaccharides by the use of a 1 kDa-dialysis membrane. After this, desalted samples were hydrolyzed with hydrochloric acid at a final concentration of 0.8 M for 20 h at 100°C. Concentrations of total combined glucose, galactose, arabinose, mannose, xylose, fucose, rhamnose, galactosamine, glucuronic acid and galacturonic acid were detected (Engel and Händel, submitted). The sum concentration is referred to as total polysaccharides. Replicate measurements revealed a standard deviation of <5% for the analytical method.

For the analysis of chlorophyll *a*, seawater samples were filtered onto GF/F filters that were stored at −20°C. According to Yentsch and Menzel (Yentsch and Menzel, 1963), chlorophyll *a* was extracted with 90% acetone in the dark and measured fluorimetrically.

Concentrations of nitrate and nitrite (sum concentration referred to as NO_x) were measured colorimetrically according to Grasshoff *et al.* (Grasshoff *et al.*, 1983) after filtration through 0.4 μm polycarbonate filters (Nucleopore).

Bacterial uptake rates and biomass production

Rates of bacterial glucose and amino acid uptake as well as BBP were determined by the use of different radiotracers, which were labelled either with [^{3}H] or [^{14}C]. For all assays, samples were incubated in the dark at a temperature of 10°C that was close to *in situ* conditions of 8−14°C.

The bacterial uptake of glucose and amino acids was estimated from the assimilation of [^{3}H]-glucose (SA 20 Ci mmol^{-1}) and an amino acid mixture labelled with [^{14}C] (SA 35 mCi mg C^{-1}), respectively, during the field study in 2007. The amino acid mixture included 15 amino acids that mimic the composition of algal protein hydrolysate. For both radiotracers, 10 mL of sample were incubated with a final concentration of

1 nmol L^{-1} for 1–2 h, depending on the bacterial activity. Incubations were stopped by the addition of formalin at a final concentration of 4%. Formalin-killed controls were run for each sampling. Samples were further processed according to the microcentrifuge method described by Smith and Azam (Smith and Azam, 1992). Briefly, three subsamples of 2 mL were centrifuged at 14 000 *g* for 20 min to obtain cell pellets that were washed two times with ice-cold 5% trichloroacetic acid. The supernatant was carefully removed and scintillation cocktail (Ultima Gold AB, Perkin Elmer) was added. After that, samples were radio-assayed by liquid scintillation counting. Replicates revealed a mean standard deviation of 5% for both assays.

BBP was estimated from the incorporation of [^{3}H]-thymidine (SA 70 Ci $mmol^{-1}$) during the field study in 2006. In 2007, BBP was assessed from the incorporation of [^{14}C]-leucine (SA 324 mCi $mmol^{-1}$). The radiotracer was added to 10 mL of sample at a saturating final concentration of 10 nmol L^{-1}. Samples were incubated for 45 min to 2 h. Further sample processing and analysis were accomplished similar to assays for bacterial uptake rates that are described above. To calculate BBP from [^{3}H]-thymidine incorporation, a conversion factor of 1.74×10^{18} cells mol^{-1} incorporated thymidine was assumed (Kirchman, 1992). Rates of [^{14}C]-leucine incorporation were converted into BBP applying the factor of 1.08×10^{17} cells mol^{-1} leucine incorporated (Kirchman, 1992). Furthermore, for both radiotracer assays a carbon conversion factor of 10 fg carbon $cell^{-1}$ was used (Caron *et al.*, 1995). Replicates revealed a standard deviation $<10\%$.

Activity of hydrolytic extracellular enzymes

The hydrolytic activity of the polysaccharide-degrading extracellular enzymes α-glucosidase and β-glucosidase and of the protein-degrading extracellular leucine-aminopeptidase were determined by the use of fluorogenic substrate analogues during the field study in 2007 (Hoppe, 1983). 4-Methyl-umbelliferyl-α-D-glucopyranosid, 4-methyl-umbelliferyl-β-glucopyranosid and L-leucyl-4-methylcoumarinyl-7-amide, respectively, were added to 200 µL of sample at a final concentration of 1 µmol L^{-1} that corresponded to the magnitude of *in situ* concentrations. Fluorescence was measured with a plate reader (Fluostar Optima, BMG Labtech) immediately after the addition of the substrate analogues and after 3 h of dark incubation. Similar to radiotracer assays, an incubation temperature of 10°C was applied. Wavelengths of 355 and 460 nm were chosen for excitation and the measurement of fluorescence emission, respectively. Calibration of relative fluorescence units was carried out with methylumbelliferone and 7-amino-4-methyl-coumarine standard solutions. Analysis of three replicates revealed a mean standard deviation of 18, 15 and 19% for α-glucosidase, β-glucosidase and leucine-aminopeptidase, respectively.

Culture experiment

In addition to the field studies, a culture experiment with *Emiliania huxleyi*, a dominant bloom-forming species in the northern Bay of Biscay, was conducted to compare the degradation rates of polysaccharides determined by ion chromatography with those calculated from glucosidase activity. For this purpose, organic matter derived from cultures of *E. huxleyi* was degraded in the dark by a natural bacterioplankton community of the North Sea. Continuous cultures of *E. huxleyi* (strain PML B92/11) were grown for 12 days in sterile-filtered seawater supplied with 30 µmol L^{-1} nitrate and 1 µmol L^{-1} phosphate at a dilution rate of 0.1 d^{-1}. A 16/8 h light/dark cycle and a photon flux density of 300 µmol photons m^{-2} s^{-1} were applied. After 12 days, the bacterial inoculum was added and the flow-through was stopped. After that, incubations were kept in permanent dark at 14°C for 14 days. The experiment was conducted at two different pCO_2 levels of 300 and 550 µatm, respectively. These values reflect the pCO_2 range determined during the two campaigns in 2006 and 2007. After 0, 2, 4, 7 and 14 days of bacterial degradation in the dark, concentrations of total polysaccharides and combined glucose as well as rates of extracellular α- and β-glucosidase were determined. The analysis of polysaccharides and glucosidase rate measurements were conducted similarly to the protocols used during field sampling.

Calculations

Rate constants of extracellular enzymes ($k_{\alpha\text{-glu},\ \beta\text{-glu},\ \text{leu}}$, d^{-1}) were calculated from reaction velocities of α-glucosidase, β-glucosidase and leucine-aminopeptidase during incubations with fluorogenic substrate analogues at a concentration of 1 µmol L^{-1} according to

$$k_{\alpha-\text{glu},\,\beta-\text{glu},\,\text{leu}} = \frac{r}{s} \quad (1)$$

where r is the enzymatic rate (µmol L^{-1} d^{-1}) and s the substrate concentration (µmol L^{-1}).

The degradation rate of combined glucose (d_{cglu}, nmol glucose L^{-1} d^{-1}) was calculated according to

$$d_{\text{cglu}} = k \cdot c_{\text{cglu}} \quad (2)$$

where $k\,(d^{-1})$ is the rate constant of extracellular β-glucosidase and c_{cglu} the concentration of total combined glucose (nmol monomer equivalent L^{-1}). The degradation rate of combined glucose represents the release of glucose monosaccharides from enzymatic polysaccharide hydrolysis per unit time. The degradation rate d_{cglu} can be converted into carbon units (nmol C L^{-1} d^{-1}) by multiplication by 6.

During the culture experiment concentrations of combined glucose were determined by HPAEC-PAD at five timepoints during 14 days of incubation. The loss of combined glucose (L_{cglu}, nmol L^{-1}) could be calculated by

$$L_{cglu} = c_{cglu(tx)} - c_{cglu(tx-1)} \quad (3)$$

where $c_{cglu(tx)}$ and $c_{cglu(tx-1)}$ (nmol L^{-1}) are the concentrations of total combined glucose at timepoint x and the timepoint before, respectively.

The loss rate of combined glucose (l_{cglu}, nmol L^{-1} h^{-1}) can be determined by

$$l_{cglu} = \frac{L_{cglu}}{t} \quad (4)$$

where L_{cglu} (nmol L^{-1})is the loss of total combined glucose and t (h) the time period. The quotient l_{cglu} is also a measure for the release of glucose monosaccharides from polysaccharide hydrolysis per time.

The glucose incorporation potential [GIP, pmol glucose (nmol biomass-C)$^{-1}$] of the bacterioplankton community was determined by

$$GIP = \frac{u_{glu}}{BBP} \quad (5)$$

where u_{glu} (pmol glucose L^{-1} h^{-1}) is the uptake rate of ^{3}H-glucose at a concentration of 1 nmol L^{-1} and BBP the bacterial biomass production (nmol C L^{-1} h^{-1}).

Similarly, the incorporation potential for amino acids [AIP, pmol amino acid (nmol biomass-C)$^{-1}$] was assessed by

$$AIP = \frac{u_{AA}}{BBP} \quad (6)$$

where u_{AA} (pmol amino acids L^{-1} h^{-1}) is the uptake rate of amino acids at a concentration of 1 nmol L^{-1} and BBP the bacterial biomass production (nmol C L^{-1} h^{-1}).

GIP and AIP represent the ability of the bacterioplankton community to convert carbon derived from glucose and amino acids, respectively, into bacterial biomass.

In this study, two conceptual approaches that assess the glucose flux from polysaccharides through the bacterioplankton community, referred to as the pool model and the flux model, are compared. The classical approach, referred to as the pool model, estimates the share of assimilated glucose from glucose uptake rates and concentrations of glucose monosaccharides (Fig. 2A). An alternative concept, referred to as the flux model, includes polysaccharide hydrolysis by extracellular glucosidase that provides glucose for bacterial uptake (Fig. 2B). By the use of the two models, the bacterial glucose incorporation (GI) at *in situ* polysaccharide concentrations can be estimated. GI can be derived from the pool model (GI_{PM}, nmol C L^{-1} d^{-1}) by

$$GI_{PM} = c_{free\,glu} \cdot k_{glu-uptake} \quad (7)$$

where $c_{free\ glu}$ (nmol C L^{-1}) is the concentration of glucose monosaccharides and $k_{glu\text{-}uptake}$ (d^{-1}) the rate constant of bacterial glucose uptake. In recent studies $c_{free\ glu}$ equalled 1% of glucose in polysaccharides, and was close to the detection limit of sensitive analytical tools for sugar analysis (Kirchman *et al.*, 2001; Sempéré *et al.*, 2008; Kaiser and Benner, 2009). Accordingly, $c_{free\ glu}$

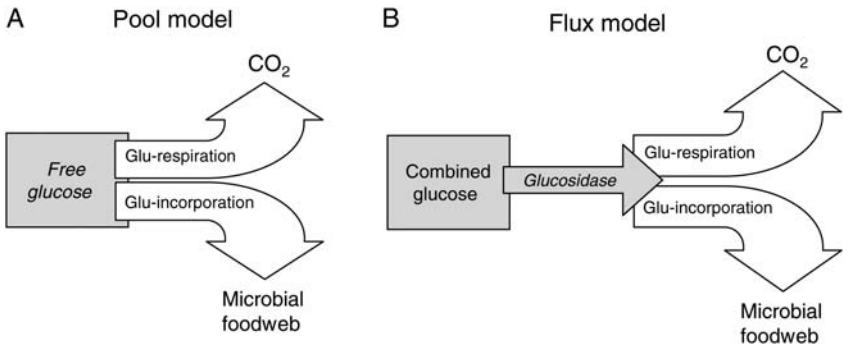

Fig. 2. Conceptual models of bacterial polysaccharide turnover. The pool model (**A**) represents the conventional pathway for calculations of polysaccharide turnover that include concentrations of glucose monosaccharides. The flux model (**B**) is not based on glucose monosaccharide concentrations. Instead, the flux of glucose monosaccharides is estimated from the polysaccharide pool by the use of extracellular glucosidase rates. See text for further explanation.

in field samples was estimated to be 1% of *in situ* concentrations of total combined glucose that were determined by HPLC-PAD. The rate constant $k_{\text{glu-uptake}}$ was calculated from glucose uptake rates at 1 nmol [^{3}H]-glucose L^{-1} by

$$k_{\text{glu-uptake}} = \frac{u_{\text{glu}}}{s} \quad (8)$$

where u_{glu} is the glucose uptake rate (pmol L^{-1} h^{-1}) and s the substrate concentration (pmol L^{-1}).

GI was also calculated from the flux model (GI_{FM}, nmol C L^{-1} d^{-1}) by

$$GI_{FM} = d_{\text{cglu}} \cdot 0.4 \quad (9)$$

where d_{cglu} (nmol C L^{-1} d^{-1}) is the degradation rate of combined glucose at *in situ* concentrations. It is assumed that glucose released by the hydrolysis of combined glucose is completely consumed by bacterioplankton, and distributed between BBP and bacterial respiration. Experimental studies have revealed that ~40% of consumed glucose was converted into bacterial biomass, while 60% was respired (Griffiths *et al.*, 1984; Rich *et al.*, 1996). Hence, a factor of 0.4 was applied to estimate the fraction of glucose that is used for the production of cellular carbon.

Statistical analysis

For calculations, statistical tests and illustration of the data the software packages Microsoft Office Excel 2003, XL STAT (Addinsoft), Sigma Plot 9.0 (Systat) and SigmaStat 3.5 (Systat) were used. Differences were tested for significance by means of *t*-test, paired *t*-test and *Wilcoxon* signed rank test, respectively, if tests for normality or equal variance failed. The significance level for all tests was $P < 0.05$. As a measure of linear dependence between two quantities, the Pearson product-moment correlation coefficient (r) was calculated.

RESULTS

The development of phytoplankton blooms in the northern Bay of Biscay

The two field studies in June 2006 and May 2007 took place after the main spring bloom that typically develops during April in this area. During the two campaigns smaller blooms could be detected as high reflectance patches in satellite images. The bloom development during the campaigns differed between the two subsequent years. In June 2006, a coccolithophore bloom at the transition from growth to the stationary phase was investigated. In particular in the northern part of the study area, which was sampled later during the cruise, a senescent bloom stage was evident. High concentrations of transparent exopolymer particles revealed intense phytoplankton exudation under nutrient-deficient conditions (Harlay *et al.*, 2009). In 2007, stations along the shelf-break were sampled ~2–3 weeks earlier in the year and a transition from a diatom bloom to a coccolithophore bloom was observed during this campaign. The high production of organic carbon by phytoplankton during the two studies is representative for this area during late spring and summer.

Bacterioplankton production during the phytoplankton blooms

Concentrations and composition of polysaccharides along the transect will be given elsewhere in more detail. Briefly, total polysaccharide concentrations in the upper water column (<50 m) ranged from 0.49 to 2.47 μmol monomer equivalent L^{-1} and showed high variability between stations and the two subsequent years (Table I). Concentrations in June 2006 were significantly higher than in May 2007 ($P = 0.028$, *t*-test). Differences in polysaccharide concentration between the 2 years can be attributed to differences in the bloom development. Most likely the accumulation of

Table I: Concentrations of combined carbohydrates in the upper water column of the northern Bay of Biscay (June, 2006 and May, 2007)

		Total combined carbohydrates		Total combined glucose	
Year	Depth (m)	μmol monomer equiv. L^{-1}	% of TOC	μmol monomer equiv. L^{-1}	% of total polysaccharides
2006	3	1.80 (1.06–2.24)	13 (7–16)	0.67 (0.34–1.27)	36 (24–57)
	30	1.52 (1.04–2.47)	na	0.48 (0.40–0.60)	34 (22–40)
	50	1.36 (0.71–2.29)	na	0.44 (0.17–0.85)	31 (13–57)
2007	3	1.19 (0.70–1.87)	7 (4–8)	0.64 (0.26–1.02)	53 (39–59)
	40	1.08 (0.49–1.94)	7 (3–12)	0.47 (0.34–0.66)	50 (22–70)

Average values as well as minimum and maximum values in brackets are given.

polysaccharides produced during growth and decline of a coccolithophore bloom resulted in higher polysaccharide concentrations in 2006. In both years, maximum concentrations were determined at stations close to the coasts of France and Ireland. Within the upper water column, mean polysaccharide concentrations for all stations showed a decrease with depth. Glucose was the dominant neutral sugar in polysaccharides and contributed up to 53% of monomers in 2007. The fraction of combined glucose in 2007 was significantly higher than in 2006, when polysaccharides contained only 31–36% of combined glucose (Table I).

Despite differences in polysaccharide concentrations, BBP revealed similar values in 2006 and 2007, ranging from 0.5 to 25.7 nmol C L^{-1} h^{-1} in the upper water column. For both years, a direct relationship between BBP and the concentration of total polysaccharides could be determined (Fig. 3). Therefore, polysaccharide concentrations can be suggested to co-determine bacterial growth in the northern Bay of Biscay during summer. BBP was also positively correlated with chlorophyll *a* concentrations in May 2007, while no significant relationship could be determined in 2006. Data for all stations sampled in 2006 can be assigned to one linear regression, while two linear functions are necessary to describe the relationship of both total polysaccharide and chlorophyll *a* concentration to BBP in 2007 (Fig. 3). This is likely related to differences in the phytoplankton bloom development during the two subsequent years. In 2006, a progressively ageing coccolithophore bloom along the transect was associated with a gradual northward decrease in polysaccharide concentration and BBP. In 2007, the temporal and spatial development of the bloom situation was patchy, since a descending diatom bloom coincided with an initial coccolithophore bloom. The regression analysis of polysaccharide concentrations and BBP shows that stations 2 and 8 separate from the other ones along the transect (Fig. 3). These two stations in the southern part of the study area were dominated by a declining diatom bloom, which was indicated by significant amounts of diatom-related pigments and phaeophytin *a* (de Bodt, 2009). Since total polysaccharide concentrations and the percentages of glucose were similar to the other stations, it seems likely that other organic matter components associated with this late diatom bloom stage were less degradable for bacterioplankton and affected BBP. With regard to chlorophyll *a*, the picture is more heterogeneous because data split into two subsets of similar size. Here, small-scale differences in species composition and physiological conditions of the phytoplankton community likely separated samples into two groups with

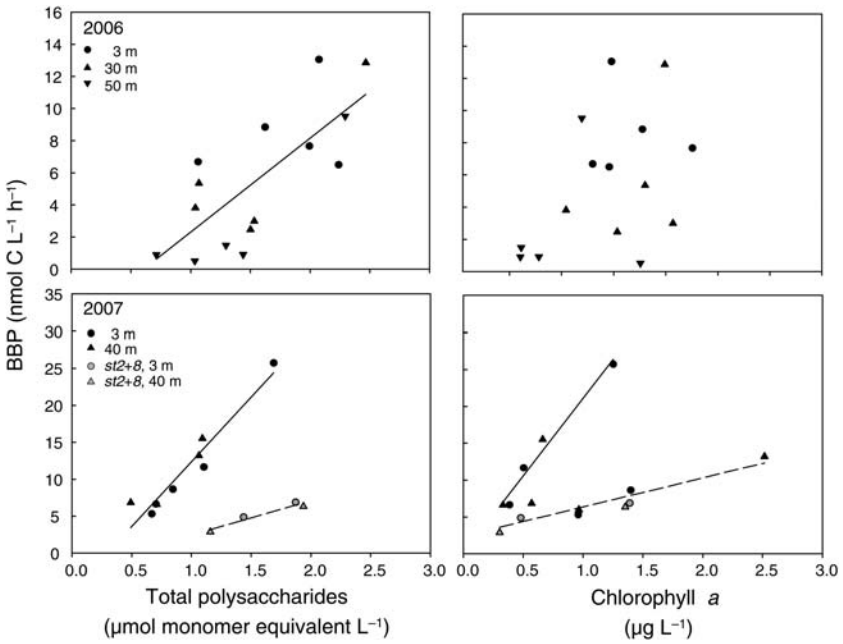

Fig. 3. Concentrations of total polysaccharides (µmol monomer equivalent L^{-1}) and chlorophyll *a* (µg L^{-1}) related to bacterial biomass production (BBP, nmol C L^{-1} h^{-1}) in 3- and 40-m depth along the transects sampled in 2006 and 2007. For both years, a significant linear relationship between total polysaccharide concentrations and BBP could be determined [2006: $r^2 = 0.57$, $P = 0.001$, $n = 15$; 2007: $r^2 = 0.93$, $P < 0.0001$, $n = 9$ (solid line), $r^2 = 0.93$, $P = 0.03$, $n = 4$ (dashed line)]. In 2007, also chlorophyll *a* concentration and BBP were significantly correlated [$r^2 = 0.98$, $P = 0.002$, $n = 5$ (solid line); $r^2 = 0.84$, $P = 0.0005$, $n = 9$ (dashed line)]. Regression analysis for 2007 includes data of one revisited station.

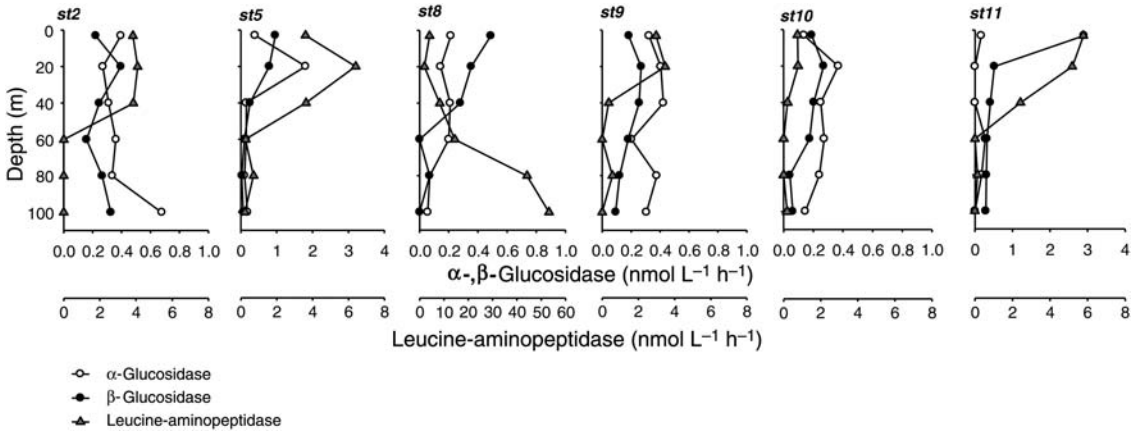

Fig. 4. Depth profiles of extracellular α-glucosidase, β-glucosidase and leucine-aminopeptidase activity (nmol L^{-1} h^{-1}) at substrate concentrations of 1 μmol L^{-1} for stations sampled in 2007. Note different scales for glucosidase activity at stations 5 and 11, and for leucine-aminopeptidase activity at station 8.

different linear regression fits for the correlation of chlorophyll *a* concentration to BBP (Fig. 3).

The enzymatic hydrolysis of polysaccharides and proteins

Activities of extracellular glucosidases and leucine-aminopeptidase were measured to track the initial step of bacterial polysaccharide and protein turnover due to enzymatic cleavage outside the bacterial cells. In May 2007, rates of leucine-aminopeptidase were on average 15 and 13 times higher than rates of α-glucosidase and β-glucosidase, respectively. In the upper 60 m of the water column glucosidase activity at a substrate concentration of 1 μmol L^{-1} ranged from 0.1 to 1.8 nmol L^{-1} h^{-1}, while leucine-aminopeptidase showed rates between 0.2 and 14.5 nmol L^{-1} h^{-1} (Fig. 4). These rates corresponded to enzymatic rate constants ($k_{\alpha\text{-glu}}$, $_{\beta\text{-glu}}$, $_{leu}$) of 0.003–0.043 d^{-1} and 0.005–0.347 d^{-1} for extracellular glucosidases and leucine-aminopeptidase, respectively. Rate constants reveal mean hydrolytic turnover times of 170 and 165 days for α- and β-glycosidic polysaccharides, and of 39 days for proteins in the upper 60 m. Rates of extracellular enzymes decreased strongly <60-m depth in most profiles. However, exceptions were found, where in particular glucosidase activity was more homogenously distributed over the water column (Fig. 4).

Along the transect, a significant correlation between glucosidase and leucine-aminopeptidase activity could not be determined. Furthermore, no direct relationship was found between glucosidase activity and concentrations of total polysaccharides and combined glucose.

A culture experiment with *Emiliania huxleyi* was conducted to test whether glucosidase rates determined by the use of fluorogenic substrate analogues could be applied to estimate the hydrolytic loss of monomers from polysaccharides. Two cultures of *E. huxleyi*, grown at 300 and 550 μatm pCO_2, produced polysaccharides with a high proportion of combined glucose (Table II), similar to polysaccharides in the northern Bay of Biscay in 2007 (Table I). Although concentrations of total polysaccharides and combined glucose in the cultures were ~10 times higher than in the northern Bay of Biscay, the carbon share included in polysaccharides accounted also for 6% of TOC, similar to polysaccharides in field samples (Tables I and II). The substrate analogues used for glucosidase assays during the field study and in the laboratory experiment contain a fluorescent marker that is bound to a glucose molecule by a glycosidic linkage. Hence, the enzymatic hydrolysis of this substrate analogue mimics the release of glucose from terminal position, and not the intramolecular cleavage of complex polymers into large subunits. Equation (2) was applied to calculate the degradation rate of glucose by the use of glucosidase rates (d_{cglu}). Results can be compared

Table II: Concentrations of combined carbohydrates in cultures of Emiliania huxleyi

	Total combined carbohydrates		Total combined glucose	
Culture	μmol L^{-1}	% of TOC	μmol L^{-1}	% of total combined carbohydrates
Ehux-300	10.9	6	7.3	67
Ehux-550	12.9	6	8.6	67

Cultures grown at 300 and 550 μatm pCO_2 are referred to as *Ehux*-300 and *Ehux*-550, respectively.

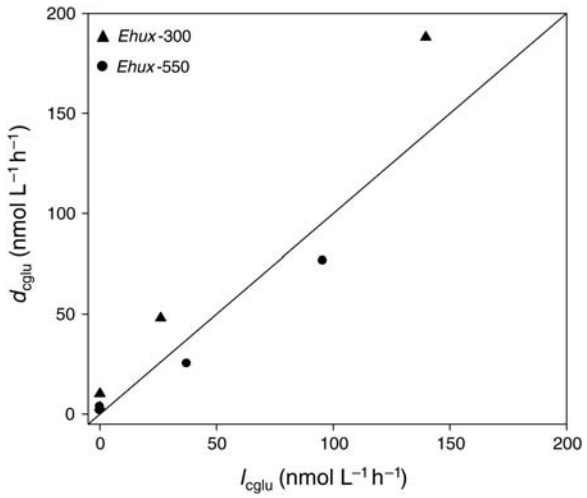

Fig. 5. Loss of combined glucose during bacterial degradation of *Emiliania huxleyi* monocultures. Organic matter was derived from cultures grown at 300 μatm (*Ehux*-300) and 550 μatm pCO_2 (*Ehux*-550). Two ways of calculation are compared: The loss rate of combined glucose (l_{glu}) was estimated by subtracting concentrations of combined glucose measured by HPAEC-PAD. Degradation rates (d_{cglu}) were calculated from rates of extracellular glucosidase and the initial concentration of combined glucose determined by HPAEC-PAD.

with loss rates that were determined by chemical analysis (l_{cglu}) and calculated according to equation (3). Estimates of d_{cglu} and l_{cglu} were in good accordance, and differed only by a factor of 1.5 on average (Fig. 5). Based on these experimental results, it can be assumed that d_{cglu} provides a reasonable estimate of the *in situ* glucose release from bacterial polysaccharide hydrolysis along the transect. Accordingly, glucose was released from polysaccharides at a mean rate of 4.9 nmol L^{-1} d^{-1}. The integration of rates over depth reveals that bacterial polysaccharide degradation sustained a carbon flux of 15.2–32.3 mg C m^{-2} d^{-1} in the upper 100 m of the water column along the shelf-break (Table III). On average 71% of the glucose release in the upper 100 m was derived from polysaccharide degradation in the upper 40 m of the water column (Fig. 6).

The bacterial utilization of sugars

The incorporation of glucose, the hydrolysate of combined glucose, was investigated in 2007. Rates at 3- and 40-m depth ranged from 3.9 to 25.8 pmol glucose L^{-1} h^{-1} at a glucose concentration of 1 nmol L^{-1}. Accordingly, the mean turnover time for glucose monosaccharides was 4.5 days and substantially longer than 0.6 days for free amino acids. Regression

Table III: Release of glucose from polysaccharides (nmol glucose L^{-1} d^{-1}) and resulting carbon fluxes integrated over 100-m depth (mg C m^{-2} d^{-1}) at stations sampled in 2007

	Depth (m)	st2	st5	st8	st9	st10	st11
Glucose release (nmol glu L^{-1} d^{-1})							
	3	3.7	13.2	11.9	1.1	1.6	39.3
	20	7.7	11.6	3.5	3.4	5.9	1.7
	40	2.8	4.1	5.5	—	2.0	3.4
	60	1.9	1.1	—	17.3	2.1	1.4
	80	3.9	0.2	1.3	1.9	0.5	1.1
	100	4.0	0.4	—	0.1	0.7	1.5
Depth-integrated carbon flux (mg C m^{-2} d^{-1})		28.3	25.4	18.5	32.3	15.2	21.2

Rates could not be calculated for three sample because either β-glucosidase rates were below the detection limit (*st8*/60 m, *st8*/100 m) or the concentration of combined glucose was not available (*st9*/40 m).

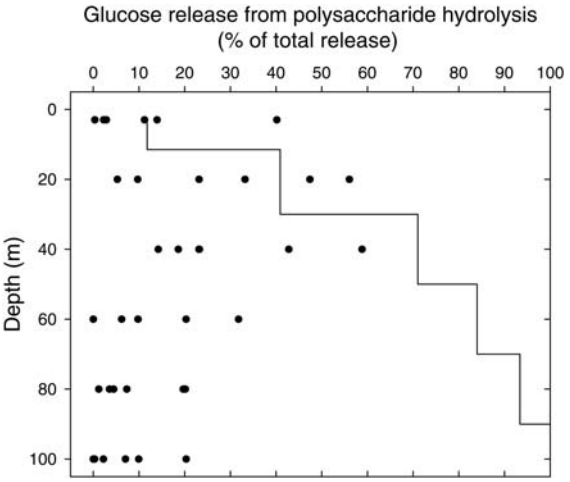

Fig. 6. Release of glucose from polysaccharides (%) over 100-m depth. Dots show the contribution of different depth layers to the total hydrolysis. The vertical steps sum up the fractions hydrolyzed in the different depth layers.

analysis revealed a direct relationship between bacterial glucose uptake and BBP, but not between amino acid uptake and BBP (Fig. 7).

The GIP was defined according to equation (5) as the ratio of glucose uptake and biomass production. GIP increased significantly with NO_x concentrations in most samples, suggesting that the share of glucose used for the build up of bacterial biomass was co-determined by NO_x availability (Fig. 8). GIP was significantly higher at 40-m than at 3-m depth (paired *t*-test, $P = 0.02$). In contrast, AIP (equation 6) was similar in both depths (Fig. 9).

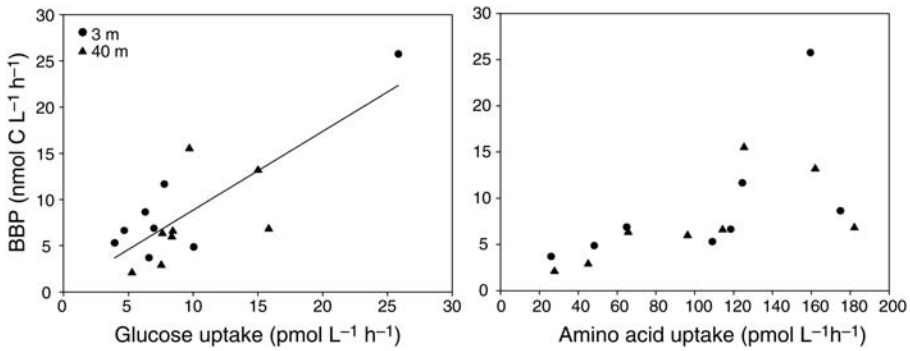

Fig. 7. Uptake rates of free glucose and free amino acids (pmol L^{-1} h^{-1}) related to bacterial biomass production (BBP, nmol C L^{-1} h^{-1}) in 3- and 40-m depth along the transect. A significant linear relationship between glucose uptake and BBP could be determined ($r^2 = 0.63$, $P < 0.001$, $n = 16$). Regression analysis includes data of two revisited stations.

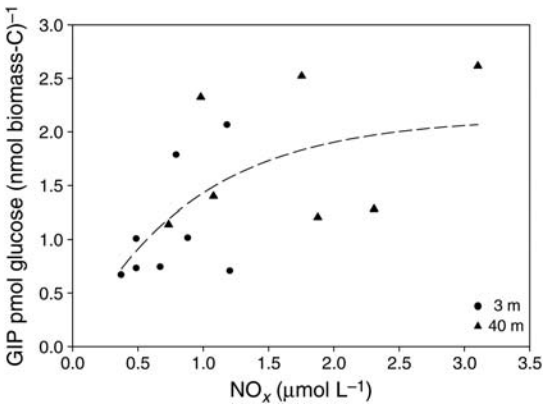

Fig. 8. The glucose incorporation potential [GIP, pmol glucose (nmol biomass-C)$^{-1}$] shown as a function of the sum concentration of nitrate and nitrite (NO_x, µmol L^{-1} h^{-1}). A significant non-linear relationship with $y = 2.14(1\text{-}e^{-1.1x})$ ($r^2 = 0.37$, $P = 0.02$, $n = 16$) could be determined. Regression analysis includes data of two revisited stations.

Glucose fluxes driven by bacterial polysaccharide turnover

Carbon fluxes driven by bacterial glucose turnover are based on two processes, the enzymatic hydrolysis of polymers and the subsequent uptake of monomeric hydrolysates. The classical concept, here referred to as the pool model, is based on the existence of a permanent monosaccharide pool (Fig. 2A) that is consumed by bacterioplankton with a certain uptake rate. However, field samples analysed by ion chromatography revealed neither in the Pacific Ocean nor in the Atlantic Ocean detectable monosaccharide concentrations (Kaiser and Benner, 2009). These findings support the assumption that the coupling of enzymatic polysaccharide hydrolysis and subsequent glucose uptake in temperate marine systems is in most cases strong enough to impede a substantial accumulation of free glucose. Therefore, we developed an alternative concept to estimate carbon fluxes from polysaccharides to bacterioplankton, which is referred to as the flux model (Fig. 2B). The flux model assumes that monosaccharides derived from polysaccharide hydrolysis are completely consumed by the bacterioplankton community and distributed between BBP and bacterial respiration on short-time-scales, so that no significant monosaccharide pool accumulates. To estimate this glucose flux, concentrations of polysaccharides and rates of extracellular glucosidase are included. We calculated the bacterial GI according to the two models (GI_{PM}, GI_{FM}) by the use of equations (7) and (9), respectively, for samples of 3- and 40-m depth. Overall, results show that the two conceptual approaches are in good agreement although highest values of GI_{FM}, which were computed for samples of station 5 (40 m) and station 8 (3 and 40 m), exceed the corresponding values of GI_{PM} (Table IV). The surface sample of station 11 represents an outlier, since GI_{FM} was 18 times higher than GI_{PM}. Station 11 is closest to the coast of Ireland and extracellular glucosidase activity in this sample was about eight times higher than on average along the transect. It can be suggested that the input of allochtonous material induced high glucosidase activity that was not capable of hydrolyzing organic matter of terrigenous origin efficiently. Hence, the estimate of glucose release by the use of glucosidase rates would not be valid at this coastal station. For all other stations, GI_{FM} computed reliable values for bacterial GI, and should represent a valid alternative to the classical pool model. Both models allow an estimate for the portion of BBP that was supported by glucose consumption. In the pool model and the flux model, the bacterial turnover of combined glucose supported on average 5.1 and 7.6% of BBP, respectively, in the northern Bay of Biscay (Table IV).

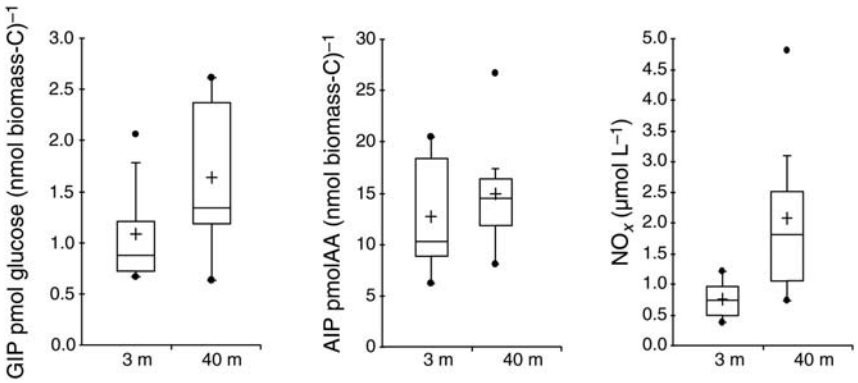

Fig. 9. Box plots of glucose incorporation potential [GIP, pmol glucose (nmol biomass-C)$^{-1}$], amino acid incorporation potential [AIP, pmol AA (nmol biomass-C)$^{-1}$] and sum concentration of nitrate and nitrite (NO_x, µmol L^{-1}) in samples collected at 3- and 40-m depth in 2007. Crosses represent mean values and dots show minimum and maximum values.

Table IV: Bacterial glucose incorporation (nmol C L^{-1} d^{-1}) calculated from the pool model (GI_{PM}) and the flux model (GI_{FM}) and percentages of bacterial biomass production (BBP) supported by carbon from glucose uptake (% glucose-C in BBP)

		Glucose incorporation (nmol C L^{-1} d^{-1})		% Glucose-C in BBP	
Station	Depth (m)	GI_{PM}	GI_{FM}	Pool model	Flux model
2	3	10.1	8.9	8.6	7.6
	40	5.1	6.7	7.4	9.7
5	3	6.4	1.2	2.3	0.4
	40	5.0	9.8	10.0	19.6
8	3	10.2	28.7	6.2	17.3
	40	9.0	13.2	5.9	8.7
9	3	1.5	2.7	1.1	2.2
	40	—	—	—	—
10	3	2.3	3.8	1.5	2.4
	40	5.0	4.8	3.1	3.0
11	3	5.1	94.3	2.5	45.5
	40	7.9	8.1	4.8	5.0

DISCUSSION

Methodological considerations

The most abundant storage polysaccharide in marine phytoplankton is laminarin, a β-glucan of ~4 kDa size (Meeuse, 1962; Painter, 1983). Polysaccharides in the surface ocean contain high fractions of glucose. They are often freshly produced by phytoplankton and thus not diagenetically altered (Rich *et al.*, 1996; Skoog *et al.*, 1999, 2002; this study). Therefore, it can be assumed that the share of laminarin in surface waters is generally high. It was shown that glucose monosaccharides contribute a high share to the hydrolysate of laminarin at non-saturating concentrations because extracellular glucosidases have higher affinities than glucanases to laminarin at low concentrations (Alderkamp *et al.*, 2007). It must be assumed that extracellular glucosidase activity generates the flux of glucose monosaccharides from laminarin, and also drives the hydrolytic turnover of combined glucose at natural concentrations in seawater. This is strongly supported by our culture experiment that investigated the bacterial degradation of glucose in polysaccharides produced from *E. huxleyi*. Results revealed that the loss of combined glucose in the freshly produced organic matter can be inferred from rates of extracellular β-glucosidase (Fig. 5). Therefore, the use of extracellular glucosidase rates determined with simple fluorescent substrate analogues according to Hoppe (Hoppe, 1983) in combination with the precise chemical detection of combined glucose concentrations is a useful tool to quantify the release of glucose monosaccharides from glucose-rich, reactive organic matter. In particular during field studies,

degradation rates derived from the combination of polysaccharide analysis with measurements of extracellular glucosidase activity can be used to estimate the *in situ* flux of glucose that is available for bacterioplankton consumption.

Organic matter sources for bacterioplankton productivity in the Bay of Biscay

The production of biomass by heterotrophic bacterioplankton in the ocean is regulated by a multitude of interacting factors such as organic matter availability and composition, inorganic nutrient concentrations, temperature and mortality due to grazing and viral infections (Bratbak *et al.*, 1992; Ducklow, 1999; Vazquez-Dominguez *et al.*, 2005). Values of BBP in the northern Bay of Biscay during late spring were more representative for mesotrophic inshore regions than for oligotrophic offshore sites in the Atlantic Ocean (Hoppe *et al.*, 2006), and similar to values published for shelf-break stations in the southern Bay of Biscay (Cantabrian Sea) during the same season (Barquero *et al.*, 1998). BBP in the northern Bay of Biscay increased significantly with increasing concentrations of polysaccharides (Fig. 3). This direct relationship between BBP and polysaccharide concentration was found in 2006 and in 2007, despite the fact that the two campaigns were carried out during different phases of phytoplankton blooms. In June 2006, a coccolithophore bloom at the transition from growth to the stationary phase was sampled and higher concentrations of polysaccharides and a higher polysaccharide share in TOC than in 2007 pointed to the accumulation of semi-labile organic matter (Table I). BBP in June 2006 was not correlated with chlorophyll *a* concentrations, suggesting that bacterioplankton growth was more strongly supported by accumulated organic carbon, including polysaccharides, than by labile compounds during this late bloom phase. In May 2007, samples along the transect were collected after a diatom bloom and during an actively growing coccolithophore bloom. At this time, BBP was also positively correlated with chlorophyll *a* (Fig. 3). Therefore, it can be assumed that BBP was substantially supported by carbon freshly produced from coccolithophores that contained polysaccharides but also higher shares of labile compounds such as amino acids. The tight coupling of BBP to polysaccharide concentrations during both years suggests that bacterial growth was affected by the availability of semi-labile organic matter. A previous study conducted in the southern Bay of Biscay analysed BBP in profiles up to 60-m depth. Here, maximum BBP did not coincide with maximum chlorophyll *a* concentrations at most stations (Barquero *et al.*, 1998). This might suggest that the vertical distribution of semi-labile organic matter in the euphotic zone can also impact bacterial growth in the Bay of Biscay.

The degradation of semi-labile organic matter by hydrolytic extracellular enzymes

Marine bacterioplankton communities produce a spectrum of extracellular enzymes that acquire organic compounds for bacterial consumption by the hydrolysis of organic polymers in seawater (Hoppe *et al.*, 1988). Synthesis and activities of extracellular enzymes are regulated by environmental factors. Changes in substrate concentrations, temperature, pH and salinity on small spatial and temporal scales induce highly variable rates (Chróst, 1991). Therefore, ranges of extracellular enzyme activities that are characteristic of different provinces in the temperate ocean can hardly be determined. Rates of extracellular glucosidases and leucine-aminopeptidase determined in the Bay of Biscay were in the broad range of values published for various coastal and offshore systems in the temperate ocean (Fig. 4). Similar to most previous studies, leucine-aminopeptidase activity was higher than glucosidase activity. This was explained by higher constitutive aminopeptidase activities that enable bacterioplankton communities to efficiently exploit proteins during short pulses of organic matter input (Christian and Karl, 1998). In our study, neither along the transect nor in vertical profiles could correlations between glycolytic and proteolytic activities be determined. This reveals the decoupling of bacterial polysaccharide and protein degradation during summer, and suggests a direct regulatory effect of organic matter composition on enzymatic rates. Concentrations of combined glucose and rates of extracellular glucosidase were not correlated along the transect, suggesting that *in situ* polysaccharide concentrations were more closely related to phytoplankton production than to bacterial degradation activity during our study.

In most depth profiles, rates of extracellular enzymes decreased <60 m (Fig. 4). Given that temperature changes between 60 and 100 m were smaller than 0.1°C and on average 1.8°C lower than sea surface temperature (SST), it can be assumed that the water column was consistently stratified during our study. Enzyme activities below the thermocline were likely directly affected by the decrease in temperature, but also by lower bacterial abundances and decreased substrate concentrations. Furthermore, the bacterioplankton communities near the surface and <60 m might have specific metabolic capacities and specific enzyme profiles that allow growth

in environments with different qualities and quantities of organic matter. Overall, our results reveal a regulation of extracellular enzyme activities in the Bay of Biscay by several interacting abiotic and biotic factors such as production and composition of organic matter, temperature and adaptation of the bacterioplankton community.

The release of free glucose from enzymatic polysaccharide degradation

The breakdown of polysaccharides by extracellular glucosidase generates the flux of glucose monosaccharides that is available for bacterial uptake. The range of polysaccharide concentrations was smaller than that of enzymatic rates determined at a defined substrate concentration of 1 μmol L^{-1} (Fig. 4). This demonstrates that changes in polysaccharide degradation rates were predominantly driven by variation in glucosidase rate constants and not by differences in polysaccharide concentrations. The coincidence of high enzymatic rate constants and high polysaccharide concentrations led to maximum polysaccharide degradation rates at 3- to 40-m depth (Fig. 6). Glucose monosaccharides released by polysaccharide hydrolysis were consumed on the time-scale of days. This is in line with many previous studies that identified free glucose in seawater as a labile compound (Rich *et al.*, 1996; Skoog *et al.*, 1999, 2002). Therefore, it can be assumed that a tight coupling between polysaccharide hydrolysis and bacterial glucose uptake assured a rapid metabolization of sugars derived from polysaccharide degradation by the bacterioplankton community. Owing to this tight coupling the release of glucose from enzymatic polysaccharide hydrolysis should approximately equal bacterial glucose consumption. It has been shown that the potential carbon flux mediated by bacterial glucose utilization equalled 1.1% of the photosynthetic carbon production in the upper water column of the tropical North Atlantic (Bianchi *et al.*, 1998). During our study in the Bay of Biscay, gross primary production ranged from 358 to 2375 mg C m^{-2} d^{-1} with the minimum achieved at station 5 and the maximum value determined at station 11. Given these values, the bacterial glucose release would be equivalent to 0.9 and 7.1% of autotrophic carbon fixation at station 5 and 11, respectively (Table III). Hence, the bacterial recycling of polysaccharides provided a significant carbon flux along the shelf-break.

The significance of nitrogen for bacterial sugar metabolization

Amino acids contribute a substantially higher share of carbon to the production of bacterial biomass than sugars in many different marine systems (e.g. Rich *et al.*, 1997; Kirchman *et al.*, 2001; Grossart and Simon, 2002). Also in the Bay of Biscay uptake rates of free amino acids were higher than those of free glucose, suggesting that amino acids were preferentially used for BBP (Fig. 7). The preferred utilization of amino acids can be attributed to less energy loss and lower metabolic costs for the build-up of biomass from organic nitrogen (Russell and Cook, 1995; Vallino *et al.*, 1996).

The conversion of glucose into bacterial biomass increased significantly with NO_x concentrations in most samples collected along the transect (Fig. 8). It is likely that the conversion of carbon from polysaccharides into bacterial biomass was enhanced by the use of inorganic nitrogen for *de novo* synthesis of cellular proteins. An increase in GIP with rising NO_x concentrations is consistent with previous studies, which show that the addition of inorganic nitrogen to incubation experiments and high nitrate concentrations in seawater can increase glucose assimilation by bacterioplankton (Bianchi *et al.*, 1998; Skoog *et al.*, 2002). However, it must be assumed that NO_x availability was only one factor among others that affected GIP in the Bay of Biscay. It is also likely that the carbon, nitrogen and energy supply from various other organic compounds as well as cell-physiological characteristics like energetic costs for maintenance and growth had a large influence on the bacterial utilization of glucose.

Heterotrophic bacterioplankton and autotrophic microorganisms compete for NO_x in the surface ocean, in particular when organic nitrogen sources for bacterioplankton are limited. During our study both NO_x concentrations and GIP at 40-m depth were higher than near the surface (Fig. 9). Assuming a direct effect of NO_x availability on GIP one might suggest that differences in NO_x concentrations also have the potential to establish a vertical zonation of bacterial glucose utilization. Upwelling events frequently punctuate thermal stratification during summer and introduce high concentrations of inorganic nitrogen into the upper water column of the Bay of Biscay (Pingree *et al.*, 1982; Huthnance *et al.*, 2001; Wollast and Chou, 2001). So far, only secondary effects on bacterioplankton due to increasing organic matter production after upwelling were considered. A previous study, however, showed that BBP increased immediately after an upwelling pulse, despite the fact that the production of fresh DOC by phytoplankton had not increased at that time (González *et al.*, 2003). It can be suggested that the use of inorganic nitrogen by bacterioplankton enhanced the utilization of carbon residues in the surface waters. The bacterial turnover of organic carbon accumulated during spring blooms is considered to maintain net

heterotrophy in the Bay of Biscay during summer (Serret *et al.*, 1999). Hence, the use of inorganic nitrogen for protein synthesis might allow bacterioplankton to directly benefit from upwelling events and to boost a net heterotrophic carbon balance.

The relevance of polysaccharide turnover for bacterioplankton growth

The incorporation of glucose contributed 0.4–19.6% of carbon to BBP in the Bay of Biscay during late spring (Table IV). To the best of our knowledge, these are the first estimates for the fraction of BBP supported by glucose in the eastern North Atlantic. In comparison with data published for other marine systems, average values and the high variability determined in the Bay of Biscay are most similar to the North Pacific, where glucose assimilation sustains 0.3–16% of BBP (Skoog *et al.*, 2002). Relative to the eastern North Atlantic and the North Pacific, glucose supports a higher fraction of 15–30% BBP in the Equatorial Pacific, but a lower percentage of 1–10% in the Gulf of Mexico (Skoog *et al.*, 1999) and 0.2–6% in the Ross Sea (Kirchman *et al.*, 2001). Instead of glucose, amino acids have been shown to supply the main fraction of BBP in various marine systems (Fuhrman and Ferguson, 1986; Kirchman *et al.*, 2001; Skoog *et al.*, 2002). Also during our study, extracellular leucine-aminopeptidase activity and amino acid uptake rates were clearly higher than rates of extracellular glucosidase and glucose uptake, respectively (Fig. 4). Therefore, it seems likely that proteins and amino acids were also primary substrates for bacterioplankton production in the Bay of Biscay during late spring. It appears that the main function of polysaccharides for bacterioplankton growth in the ocean is not to support a high fraction of the bacterial carbon demand. Nevertheless, BBP in the Bay of Biscay was correlated with polysaccharide concentrations. Therefore, it can be assumed that bacterial growth was dependent on the carbon amendment provided by polysaccharide turnover. Furthermore, it seems likely that carbon derived from polysaccharides flexibly supported other requirements of bacterial growth such as energy generation and *de novo* synthesis of proteins to extents that were adjustable to environmental conditions. Polysaccharide turnover times of weeks to months ensured a continuous carbon supply, in contrast to the sporadic availability of labile compounds like amino acids. Hence, carbon derived from polysaccharide turnover complements bacterial growth in the ocean when more labile substrates are available, but it also provides a reliable long-term carbon source that sustains bacterioplankton communities on time-scales of months.

In the present study, an alternative approach was proposed to quantify the bacterial polysaccharide turnover and to estimate glucose fluxes to the bacterioplankton community (Fig. 2). This concept, referred to as the flux model (Fig. 2B), quantifies the release of glucose monosaccharides from polysaccharide hydrolysis by the use of extracellular glucosidase rates, and assigns it either to incorporation or respiration. Hence, the flux model emphasizes the step of enzymatic polysaccharide hydrolysis. Overall, rates of GI calculated from the two models are in good agreement. The highest rates derived from the flux model exceed those calculated by the pool model for the same sample (Table IV). This suggests that fluxes from polysaccharide hydrolysis can substantially increase the amount of glucose available for bacterial uptake. Hence, calculations according to the flux model are in particular useful, when high polysaccharide concentrations and high extracellular glucosidase rates lead to high rates of polysaccharide hydrolysis.

Consequences of bacterial polysaccharide processing for food-webs and biogeochemical processes

The biological reactivity of polysaccharides resulted in considerable carbon fluxes in the Bay of Biscay, leading to direct implications for microbial food-webs and biogeochemical processes.

DOC escapes the grazing food chain and is almost exclusively available for bacterioplankton uptake (Pomeroy, 1974; Azam, 1998). Therefore, the release of dissolved sugars from organic particles by extracellular glucosidase activity decreases the share of organic carbon that is directly accessible for higher trophic levels. Instead, an intense bacterial degradation of particulate polysaccharides as in the Bay of Biscay may emphasize the microbial loop. Finally, the efficiency at which glucose is incorporated into bacterial biomass determines the share of carbon from polysaccharides that can be reintroduced to microbial food-webs by the ingestion of bacterial cells. Among other factors, the availability of inorganic nitrogen can enhance the conversion of carbon from sugar uptake into bacterial biomass in the Bay of Biscay as well as in other oceanic regions (Bianchi *et al.*, 1998; Skoog *et al.*, 2002).

In the marine carbon cycle, enzymatic polysaccharide hydrolysis and the rapid consumption of glucose can decrease the fraction of organic carbon available for export by particle sinking and convective mixing (Smith *et al.*, 1992; Carlson *et al.*, 1994). In particular during phytoplankton blooms, when particulate and dissolved carbon with high-polysaccharide fractions is produced (Baines and Pace, 1991; Handa *et al.*, 1992),

the short-term response of bacterial extracellular glucosidase activity to substrate input may affect the flux of carbon from the surface to the deep ocean. The efficiency of bacterial glucose assimilation determines whether carbon derived from polysaccharides gets remineralized or remains in the ocean in organic form. Respiratory CO_2 production was shown to be less time-variable than autotrophic CO_2-fixation in marine systems (Karl *et al.*, 2003). This might be attributed to the utilization of semi-labile carbon sources that resist rapid turnover, but sustain bacterial carbon requirements on time-scales of months. Hence, semi-labile organic matter, including polysaccharides, warrants constant heterotrophic activity in the ocean, and its distribution between anabolic and catabolic processes at the base of marine food-webs may affect the carbon balance of marine ecosystems.

In the near future, the ocean will experience significant changes in seawater temperature and carbonate chemistry due to increasing emissions of anthropogenic greenhouse gases. The SST of the world ocean may rise by 3–5°C during the twenty-first century (Parry *et al.*, 2007). Furthermore, the increase in CO_2 concentration predicted for the next 150 year reduces ocean pH by 0.6 units in the surface layer (Caldeira and Wickett, 2003). It has been shown that marine extracellular glucosidase activity is highly sensitive to projected changes in seawater temperature and pH. Both higher temperature and lowered pH increase the reaction velocity of extracellular glucosidases in natural marine bacterioplankton communities (Christian and Karl, 1995; Piontek *et al.*, 2009, 2010). Therefore, ocean warming and acidification are likely to affect the bacterial turnover of polysaccharides in marine systems, with potential consequences for the marine carbon cycle and the microbial loop. The accelerated enzymatic degradation of polysaccharides potentially may decrease carbon export and enhance respiratory CO_2 production in the microbial food-web of the future ocean.

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