The relationship between dissolved carbohydrates and carbohydrate-degrading enzymes in the salinity gradient of the Pomeranian Bight (southern Baltic)

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Abstract

From 1994 to 1996 changes in the concentrations of dissolved mono- (MCHO) and total dissolved polysaccharides (TCHO) as well as the activities of carbohydrate-degrading enzymes (α- and β-glucosidase, glucosaminidase) were investigated during mixing of water from the River Odra and the open Pomeranian Bight. This study addresses the question of whether their distribution was a result of physical dilution alone or if biological interactions were detectable.

Within the salinity gradient, ranging from 1.9 to 7.8 PSU, TCHO declined from 13.2 μmol l^{-1} near the Świna mouth to 2.8 μmol l^{-1} after mixing. Concentrations of MCHO decreased from 3.4 μmol l^{-1} to 1.1 μmol l^{-1} but its distribution pattern varied more between summer and autumn than that of TCHO. The hydrolysis rate (Hr) by glucosidase and glucosaminidase activities was reduced from 13.9% h^{-1} to 0.3% h^{-1} and 9.9% h^{-1} to 0.2% h^{-1}, respectively, and correlated with the uptake rate of glucose (To) by bacteria. In summer, the To/Hr ratio increased from about 1.2 to 29.4, mainly because of stronger decreases in Hr than in To. It was shown that the relationship between enzymatic release and uptake of carbohydrates influences the concentration of dissolved carbohydrates within the salinity gradient. Most probably, the decrease in carbohydrate-degrading enzymes is the result of reduced substrate stimulation and the lower number of particle-associated bacteria.

The complete text of the paper is available in PDF format at http://www.iopan.gda.pl/oceanologia/index.html

1. Introduction

The River Odra (Oder) is the largest source of nutrients entering the Pomeranian Bight (Pastuszak et al. 1996), carrying 17% of the phosphate and 15% of the fluvial nitrogen entering the entire Baltic Sea (Rosemarin et al. 1990). Besides these inorganic nutrients, the river also contains organic material in large quantities (Wedborg et al. 1994).

In most marine systems, carbohydrates constitute up to 35% of the dissolved organic carbon (DOC) (Benner et al. 1992). However, in Antarctic phytoplankton blooms, dissolved carbohydrates account for up to 50% of the semi-labile DOC pool (Kirchman et al. 2001). Carbohydrates are products of phytoplankton photosynthesis and are released by exudation, cell lysis, and microbial degradation. In addition to amino acids and lipids, they are the main components of DOC excreted by phytoplankton (Hellebust 1965, 1974). Excretion from zooplankton is a further source of dissolved carbohydrates (Klok et al. 1984, Mopper et al. 1991, Lee & Henrichs 1993). Low molecular-weight carbohydrates, such as monosaccharides, are released directly from phytoplankton into the surrounding seawater or from polymeric carbohydrates during hydrolysis by microbial enzymes. Glucose is the most abundant carbohydrate, both as a monomer as well as in polysaccharides such as glycans and cellulose (Handa & Tominaga 1969, Liebezeit & Bölter 1991, Sherr & Sherr 1999). While large amounts of low molecular weight carbohydrates are produced during periods of intensive primary production (Münster & Chrost 1990), they nevertheless remain in low concentrations in the water due to high rates of bacterial uptake (Libes 1992).

The activity of glucosidases is often used to describe the degradation of polymeric carbohydrates (Münster 1991, Vrba 1992, Hoppe et al. 1998). High β-glucosidase activity was measured during the breakdown of plankton blooms (Chrost 1991, Arrieta & Herndl 2002). Rath et al. (1993) and Hoppe et al. (1998) describe the behaviour of glucosidase activity in trophic gradients.

The investigations presented here are part of the interdisciplinary TRUMP project (transport and modification processes in the Pomeranian Bight of the Baltic Sea), carried out by the Baltic Sea Research Institute, Warnemünde, between 1993 and 1996. The main aim of the project was to describe the distribution, modification, and fate of material introduced by the River Odra, via the Szczecin Lagoon (Oderhaff), into the coastal ecosystem of the Pomeranian Bight. Physical transport and biochemical modification of different classes of material are shown over the seasonal cycle and the salinity gradient (v. Bodungen et al. 1995). The distribution of some characteristics (e.g. nutrients, chlorophyll, and particulate organic

carbon) can be described by conservative mixing processes. This pattern is not a result of conservative behaviour of the components but to a balance between auto- and heterotrophic processes (Jost & Pollehne 1998). During this study, we investigated the question of whether the distribution of dissolved carbohydrates and carbohydrate-degrading enzymes did follow a conservative mixing pattern or whether they interacted with each other and with other characteristics like phytoplankton and bacterial biomass.

2. Investigation area and methods

In July 1994, July and September 1995, and January 1996, several drift experiments and high-resolution sampling transects were undertaken in the salinity gradients of the Pomeranian Bight (Fig. 1). The objective was to describe the mixing and biological and chemical transformation of the incoming river water. The River Odra flows into the shallow Szczecin Lagoon before entering the Pomeranian Bight via the outlets Świna, Dziwna and Peene. River water and water from the bight are mixed within the lagoon, resulting in lagoon water salinities between 0.5 and 2 PSU (practical salinity units). Lagoon water enters the Pomeranian Bight in a pulse-like manner, in plumes of different sizes, and is essentially completely mixed with bight water within two or three days (v. Bodungen et al. 1995).

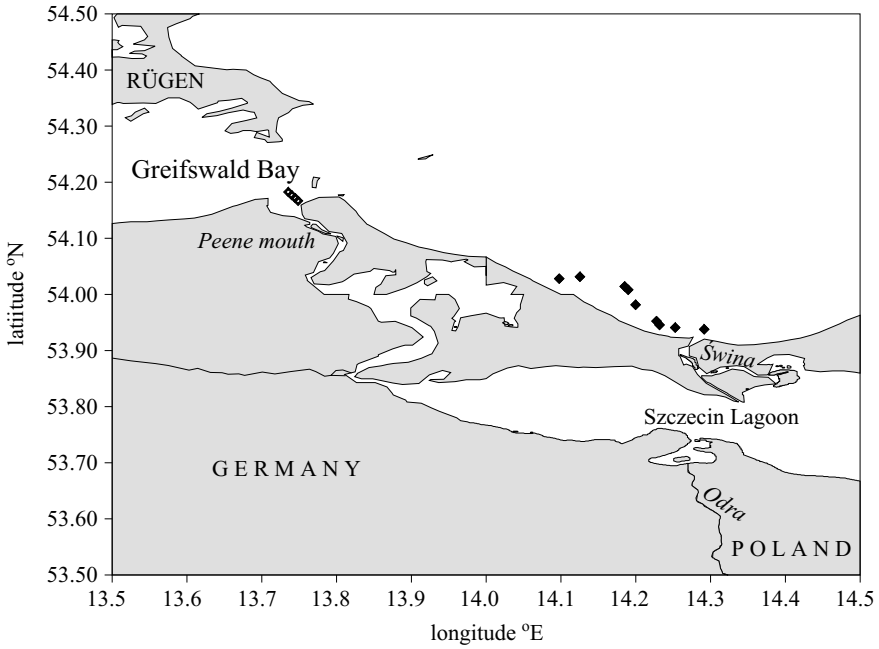

Fig. 1. Investigation area and sampling stations during drift experiments

Temperatures of 17.0 ± 1.7°C and 16.9 ± 1.1°C were measured in July 1994 and 1995, respectively. In both summers, the temperature decreased within the salinity gradient from 19.1°C to 13.2°C in July 1994 and from 18.2°C to 15.3°C in July 1995 ($r = -0.63$ and $r = -0.68$, $p < 0.01$). In September 1995 the mean value of the temperature was 13.8 ± 1.4°C and showed no significant difference within the salinity gradient. In January, temperatures ranged between –0.35°C and 0.45°C without significant change with increasing salinities.

Water samples were collected in newly introduced plumes of low salinity near the mouth of the Świna and sampling was continued until mixing with water of the open bight was complete. The frequency with which plumes were introduced into the Pomeranian Bight and the speed of mixing varied depending on meteorological conditions and so the number of samples differed in the investigation periods. Samples were taken at depths of 1–2, 5–6 and 8–10 m with a rosette sampler combined with sensors for conductivity, temperature, and density (CTD) as well as a sensor for fluorescence.

3. Analytical methods

Prior to the determination of dissolved carbohydrates, samples were filtered through precombusted GF/F filters. Dissolved monosaccharides (MCHO) were estimated in duplicate according to the 3-methyl-2-benzothiazolon-hydrazone (MBTH) method of Johnson & Sieburth (1977). For the determination of total dissolved carbohydrates (TCHO), filtered water samples were hydrolysed with 0.09N HCl (100°C, 20 h) followed by the application of the MBTH method. TCHO and MCHO concentrations are given in glucose units.

Chlorophyll *a* (chl *a*) was analysed fluorometrically (excitation at wavelength of 450 nm and emission at 670 nm) after filtration onto GF/F filters and extraction into 90% acetone (UNESCO 1994).

The degradation of carbohydrates by bacteria was assessed using α- and β-glucosidase and β-glucosaminidase activity. Glucosidase degrades mainly oligosaccharides. β-glucosaminidase acts on aminopolysaccharides like chitin or glycans (Sherr & Sherr 1999). The enzyme activities were determined according to Hoppe (1993) using the model substrates 4-methyl-umbelliferyl (MUF)-α-glucoside, (MUF)-β-glucoside and (MUF)--β-glucosaminide. To estimate the *in situ* hydrolysis of natural substrates, the hydrolysis rate (Hr [% h^{-1}]) was measured at final concentrations of 100 nM at *in situ* temperatures. Mean values of α- and β-glucosidase hydrolysis rates were used for the calculation of the ratios between turnover rate and hydrolysis rate (To/Hr).

Turnover rates (To [% h^{-1}]) of glucose were determined with D-[U-^{14}C] glucose at final concentrations of 80 nM at *in situ* temperatures (Jost & Pollehne 1998).

4. Results

Concentrations of the dissolved carbohydrates (TCHO, Fig. 2, and MCHO, Fig. 3) as well as glucosidase (Fig. 4) and glucosaminidase activities decreased as the salinity increased from 2 PSU at the Świna mouth to 6–8 PSU after dilution with water from the open bight. Other characteristics displayed a similar pattern; the distribution of chlorophyll *a* is given as an example in Fig. 5.

The highest TCHO concentrations, up to 13.2 μmol l^{-1}, and MCHO concentrations, up to 3.4 μmol l^{-1}, were measured in the growing season near the mouth of the Świna, when lagoon water entered the Pomeranian Bight. In winter, significantly lower TCHO values (2.9 to 3.9 μmol l^{-1}) and MCHO concentrations (0.3 to 1.7 μmol l^{-1}) were observed. The pattern of dilution of dissolved carbohydrates in the salinity gradient varied from year to year and between seasons. TCHO concentrations declined significantly with increasing salinities in summer and autumn (Fig. 2). In January 1996 the TCHO concentrations were approximately constant throughout the entire gradient. MCHO showed a clear relationship to salinity only in

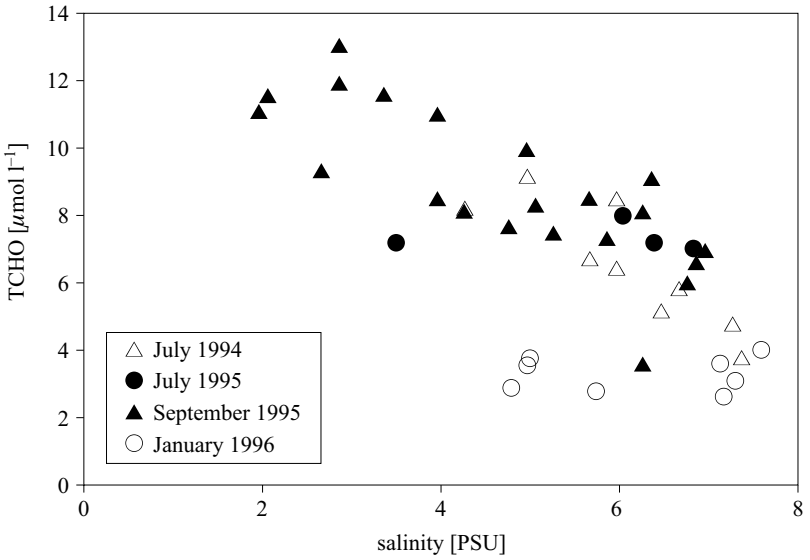

Fig. 2. TCHO concentrations in relation to salinity during the drift experiments

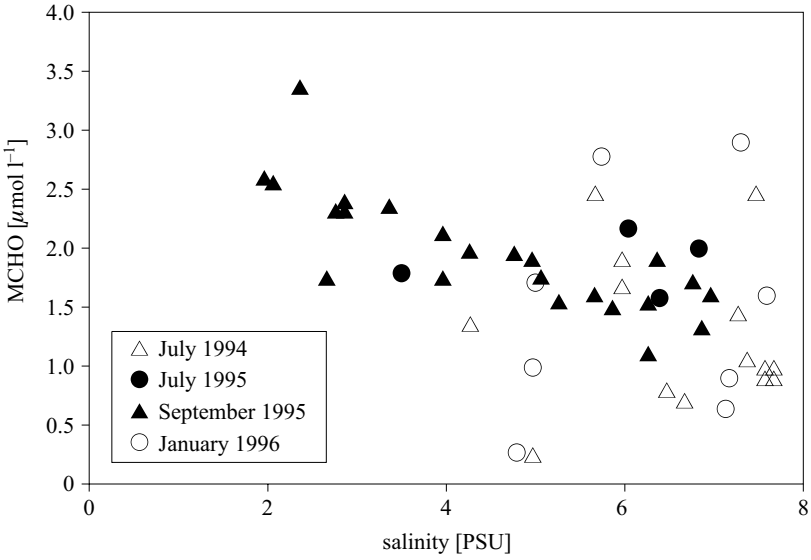

Fig. 3. MCHO concentrations in relation to salinity during the drift experiments

autumn 1995 (Fig. 3) when concentrations ranged from 3.4 to 1.1 μmol l^{-1}. During the other investigation periods, mean values of 1.4 ± 0.6 μmol l^{-1} in the summers of 1994 and 1995 and 1.5 ± 0.9 μmol l^{-1} in winter were determined.

Using all the measured values, correlation coefficients between chl *a* and TCHO, and chl *a* and MCHO of $r = 0.80$ and $r = 0.49$ ($n = 55$, $p < 0.01$) were calculated, respectively.

The hydrolysis rate of the carbohydrate-degrading enzymes α- and β-glucosidase and β-glucosaminidase decreased linearly during each investigation period during the growing season and remained at a constant low level of 0.27 ± 0.24 μmol l^{-1} h^{-1} for glucosidase activity and of 0.24 ± 0.19 μmol l^{-1} h^{-1} for glucosaminidase activity in winter. In Fig. 4, the α- and β-glucosidase activities as a function of the salinity are demonstrated. The values of the three investigated enzyme activities were in the same range. In summer, glucosidase activities fell from 13.9% h^{-1} to 0.3% h^{-1} and the glucosaminidase activity from 9.9% h^{-1} to 0.2% h^{-1} during mixing processes. While bacteria are the main carriers of these enzymes, the decrease in enzyme activities can be explained by other factors in addition to reduced bacterial abundances. Because the decrease of glucosidase activities was higher (by a factor of 46) than the decrease in bacterial counts (by a factor of 2.5), it can be deduced that the activity per bacterial cell declined. In winter, glucosidase and glucosaminidase activities amounted to only 8% of summer values.

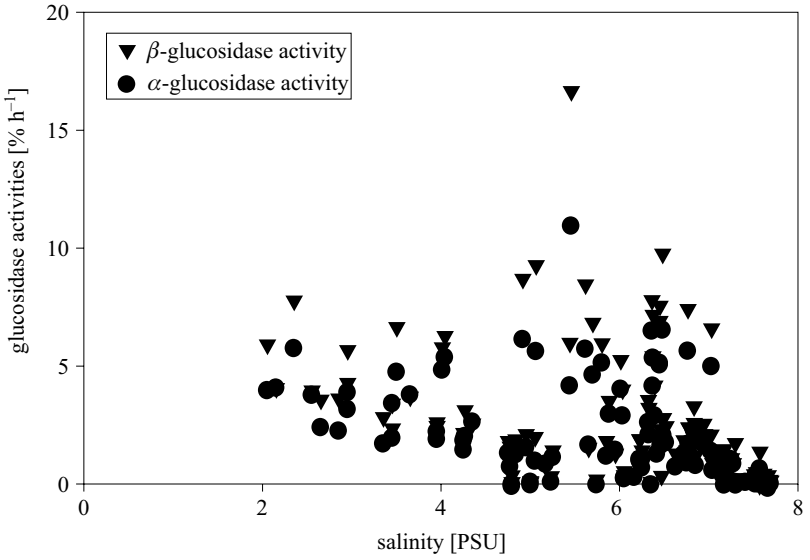

Fig. 4. α- and β-glucosidase activities in the salinity gradient between the Świna mouth and the open bight

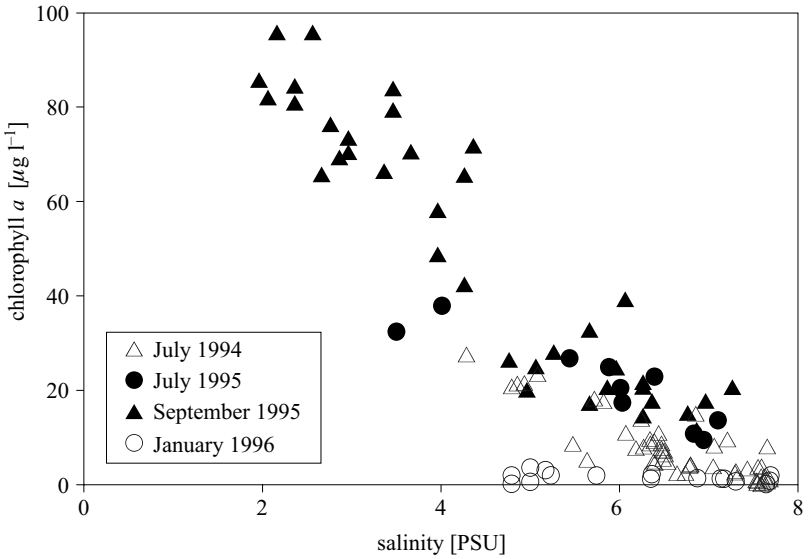

Fig. 5. The distribution of chlorophyll *a* in the salinity gradient during the investigation periods

Glucosidase and glucosaminidase activities correlated with the MCHO and TCHO concentrations (Fig. 6). These relationships were especially obvious at low enzyme activities, up to 5% h^{-1}, measured mainly at

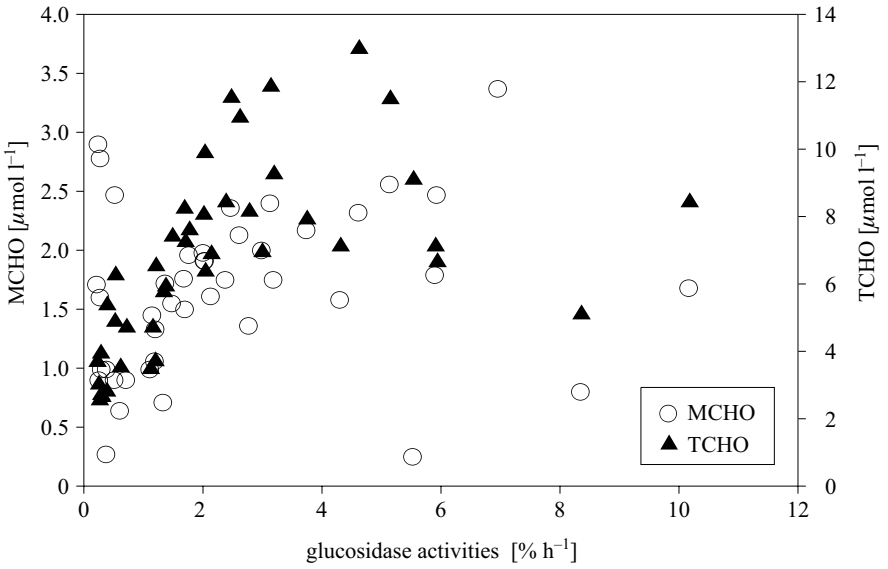

Fig. 6. Relationship between glucosidase activities and TCHO and MCHO concentrations in the salinity gradients in the Pomeranian Bight

Table 1. Correlation coefficients between dissolved carbohydrate concentrations and glucosidase and glucosaminidase activities up to 5% h^{-1} in the growing season

	MCHO [μmol l^{-1}]	TCHO [μmol l^{-1}]
glucosidase activity [% h^{-1}]	0.43	0.82
	$n = 37$, $p < 0.05$	$n = 37$, $p < 0.01$
glucosaminidase activity [% h^{-1}]	0.34	0.52
	$n = 38$, $p < 0.05$	$n = 38$, $p < 0.01$

salinities higher than 4 PSU. The correlation coefficients between the enzyme activities and TCHO were higher than between enzyme activities and MCHO (Table 1). In the outflowing lagoon water with higher enzyme activities, these relationships were not significant.

The uptake of low molecular weight substances by bacteria, determined as glucose turnover (To), was also highest in summer. At this time, glucose turnover rates up to 30% h^{-1} were measured in the outflowing lagoon water. After dilution in the salinity gradient, the values were reduced to 1–2% h^{-1}. In winter, turnover rates ranged between 0.2 and 0.5% h^{-1} only. The relationship between glucose turnover rates and glucosidase activities is demonstrated in Fig. 7.

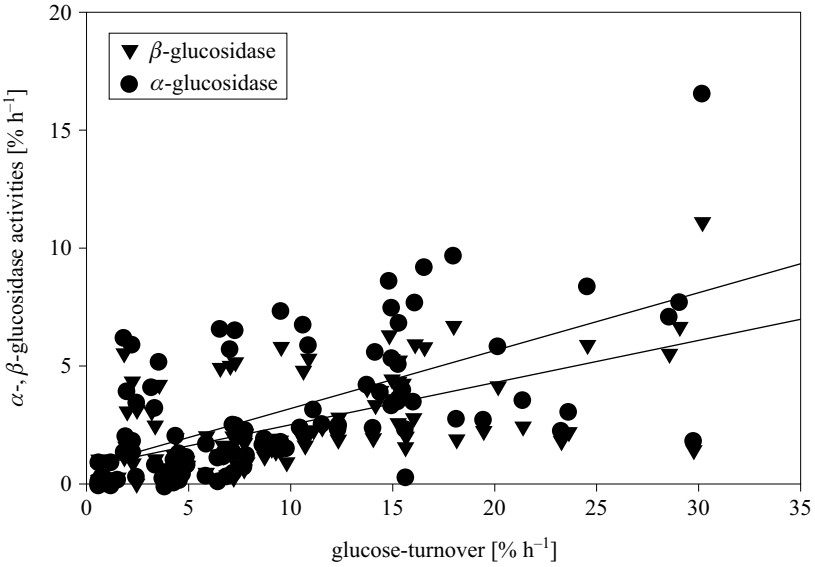

Fig. 7. Relationship between glucose turnover and glucosidase activities in the salinity gradients in the Pomeranian Bight. Correlation coefficient: $r = 0.63$, ($p < 0.01$, $n = 113$)

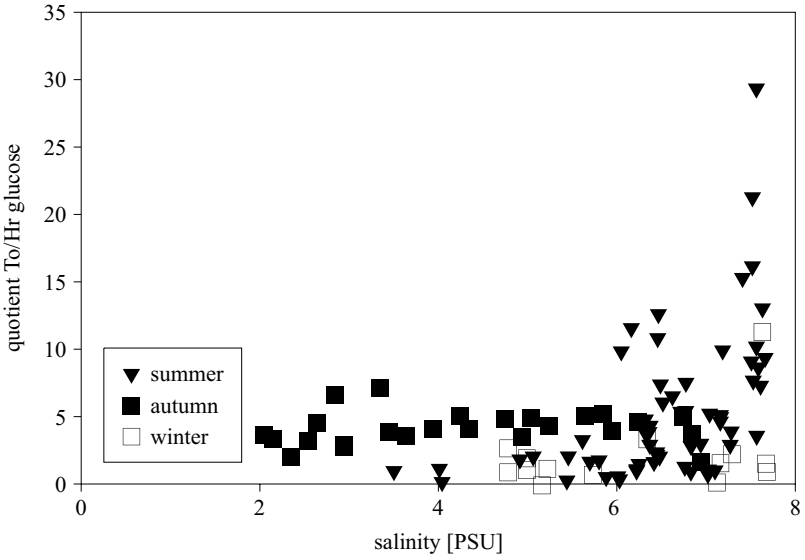

Fig. 8. To/Hr-ratios in relation to salinity in the Pomeranian Bight

The ratio between To and Hr (To/Hr) can be used as an index for the coupling of glucose uptake and release via enzymatic degradation by bacteria. In the outflowing lagoon water, median To/Hr-ratios of 1.2 were

determined in the summers, while ratios of 4.2 were measured in autumn and 1.6 in winter. In autumn and in winter, the To/Hr-ratios remained relatively constant throughout the whole salinity range. During the investigations in summer, the ratios rose to 29.4 in the open bight at higher salinities (Fig. 8). Increasing To/Hr ratios at higher salinities could be observed in both summers, but were more evident in 1994. This behaviour of the To/Hr ratios resulted from the fact that Hr was more (by a factor of 4.8) sharply reduced than To (by a factor of 2.1). No correlation of To/Hr ratios with temperature was found.

In summer and autumn, an inverse relationship between To/Hr ratios and the concentrations of TCHO and MCHO could be demonstrated (Fig. 9, Table 2). However, in autumn the relationship between the To/Hr ratio and MCHO concentrations was statistically not significant. These relationships were found in the whole gradient, but became more pronounced at salinities >5 PSU. In summer, the To/Hr ratio also correlated negatively with chl *a* ($r = -0.43$, $n = 27$, $p < 0.05$), but no significant correlation with the thymidine uptake rate, as an indicator for bacterial production, was found.

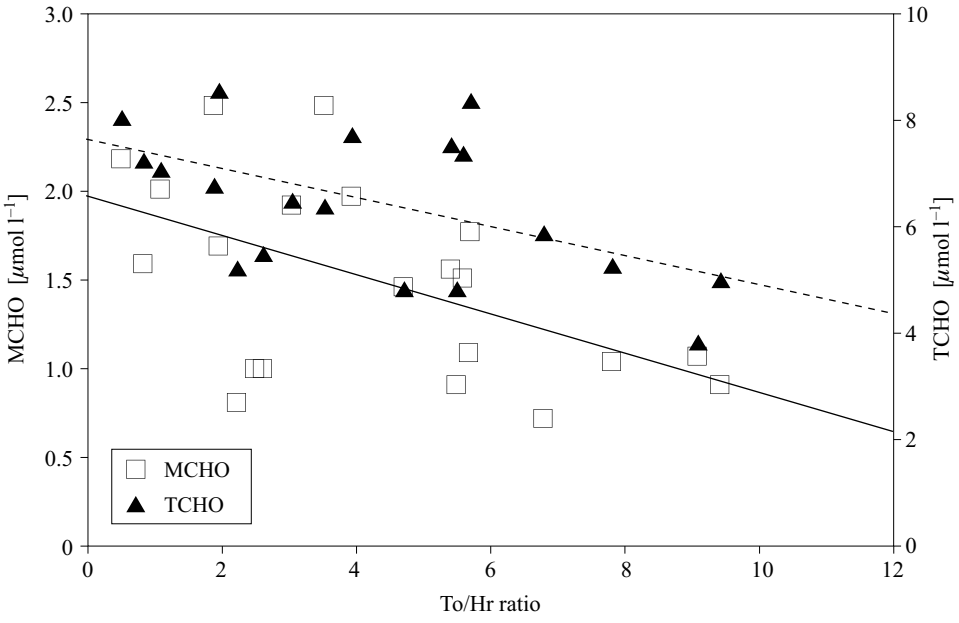

Fig. 9. The relationship between To/Hr ratios and TCHO and MCHO concentrations at salinities >5 PSU during the growth season. Correlation coefficients were: $r = -0.56$, $n = 21$, $p < 0.01$ for MCHO and $r = -0.51$, $n = 21$, $p < 0.05$ for TCHO

Table 2. Correlation coefficients between To/Hr-quotients and MCHO and TCHO concentrations in the salinity gradient in summer, autumn and winter (n.s. = not significant)

	MCHO [μmol l^{-1}]	TCHO [μmol l^{-1}]
summer	$r = -0.56$ $n = 19$, $p < 0.05$	$r = -0.46$ $n = 19$, $p < 0.05$
autumn	n.s.	$r = -0.53$ $n = 15$, $p < 0.05$
winter	n.s.	n.s.

5. Discussion

Concentrations of organic and inorganic material introduced into the Pomeranian Bight are modified by physical dilution and by transformation via biological processes. Often these processes cannot be distinguished clearly, because biological processes are masked by physical dilution. Hoppe et al. (1996) calculated the effect of physical dilution on the bacterial abundances in a tidal lagoon. Using this formula for the distribution of glucosidase activities and concentrations of dissolved carbohydrates in the salinity gradient of the Pomeranian Bight, the measured values are often higher than those obtained by physical dilution only (Table 3). These results are in agreement with Hoppe et al. (1996), who found similar differences.

Table 3. Theoretical values of dissolved carbohydrate concentrations and glucosidase activities, calculated on the basis of dilution factor, and measured values (in parentheses) in the outer range of the salinity gradient in the growing season

	Dilution factor	MCHO [μmol l^{-1}]	TCHO [μmol l^{-1}]	α-glucosidase activity [% h^{-1}]	β-glucosidase activity [% h^{-1}]
July 1994	5.5	0.1 (1.1)	1.9 (3.9)	2.3 (3.5)	1.8 (1.5)
July 1995	5.6	0.3 (2.0)	1.6 (7.1)	2.0 (3.0)	1.1 (2.1)
September 1995	1.9	1.3 (1.4)	6.2 (7.3)	1.7 (3.5)	3.1 (1.3)

In contrast, Cunha et al. (2000) found along a salinity gradient in a shallow tidal estuary (Ria de Aveiro, Portugal) that the decrease of enzyme activities followed conservative mixing.

In aquatic systems, dissolved carbohydrates, especially polymerics, are the most abundant components of dissolved organic matter (Benner 1992, Kirchman et al. 2001). Glucose and galactose are the most common of the dissolved carbohydrates in estuarine and sea water (Borch & Kirchman 1997, Biersmith & Benner 1998). In the Pomeranian Bight, measured TCHO concentrations were in the same range or higher than in other areas of the central Baltic Sea, where concentrations between 0.8 and 6.7 μmol l^{-1} were determined (Irmisch 1987). Børsheim et al. (1999) found monosaccharide concentrations between 3.0 and 50.7 μmol l^{-1} (as C) and total dissolved carbohydrate concentrations between 10 and 55 μmol l^{-1} (as C) in the surface layer of two different stations at Trondheimfjord (Norway) where salinities were between 22 and 34 PSU. On converting our results into carbon units, we found MCHO and TCHO to lie in the same range, between 6.6 and 20.4 μmol l^{-1} (as C) and 17.4 and 79.2 μmol l^{-1} (as C), respectively. However, the maximum values of MCHO were lower and the maximum values of TCHO were higher in the Baltic Sea, compared to the Trondheimfjord.

Phytoplankton are considered to be the main source of dissolved carbohydrates in water (Münster & Chrost 1990, Biersmith & Benner 1998, Kirchman et al. 2001). In the Pomeranian Bight, phytoplankton particles were the dominant source of particulate organic material over all ranges of the salinity gradient in the growth season (Jost & Pollehne 1998). Concentrations were highest in the inflowing lagoon water, because inorganic nutrients from the River Odra were converted into organic material by phytoplankton production before the water entered the bight. During our investigations, the correlations between TCHO and MCHO with chl *a* concentrations suggest that phytoplankton is also the main source for dissolved carbohydrates in this area. The closer correlation coefficient between TCHO and chl *a* can be taken as an indication that carbohydrates could be released by phytoplankton as TCHO rather than as MCHO. On the other hand, MCHO concentrations were more strongly influenced by bacterial uptake than were TCHO concentrations. This was indicated in the summer situation, when chl *a* and TCHO showed a clear relationship to the salinity and MCHO remained relatively constant. The distribution pattern of MCHO can be explained by the relationship between autotrophic and heterotrophic processes in summer, which changed in different areas of the salinity gradient. In summer, respiratory processes exceeded the primary production in the inflowing lagoon water due to light limitation,

and a negative carbon balance was calculated for the whole water column (Jost & Pollehne 1998). Due to the deeper light penetration, the carbon balance in the open bight was positive. If respiratory processes exceed autotrophic ones, the demand for low molecular weight matter by bacteria could be higher than its production, with the result that MHCO did not accumulate in the water as would be expected considering the high chl *a* concentrations. The high glucose-turnover at low salinities supports this hypothesis.

Changing To/Hr ratios can be a result of changes in the turnover rate, in the hydrolysis rates, or in both. Both activities declined in the salinity gradient. The increasing difference between the turnover and hydrolysis rates of dissolved carbohydrates, observed with increasing salinities, was mainly the result of the more rapid decrease in hydrolysis rates. The decrease of the glucosidase and glucosaminidase activities can be explained by substrate stimulation (Rath et al. 1993, Karner et al. 1995, Martinez et al. 1996) or by changes in the bacterial community. Reduced substrate stimulation was indicated by the close correlation of TCHO with glucosidase activities and with chl *a*. Arrieta & Herndl (2002) found in phytoplankton blooms that the regulation of β-glucosidase activity was driven by shifts in the bacterial community rather than by simple induction of enzyme activity. Jost & Pollehne (1998) described structural changes in the bacterial community. The percentage of particle-associated bacterial production (size fraction >2 μM) declined with increasing salinities. Particle-associated bacteria have a higher hydrolytic potential compared to free-living bacteria (Pomeroy & Wiebe 1993, Nausch 1996). Thus, those bacteria with high hydrolytic activity decreased more than the total bacterial population.

The inverse relationship between To/Hr ratios can be seen as an indication that dissolved carbohydrate concentrations declined when the uptake of glucose exceeded the glucose release from polymeric substrates. According to Jost & Pollehne (1998), the sums of autotrophic and heterotrophic processes are well balanced in the outer range of the salinity gradient. In a balanced system, a close connection exists between release and uptake of low molecular weight substrates exists (Hoppe et al. 1988). However, the increasing To/Hr ratios indicated that the demand for dissolved carbohydrate became higher than its release by enzymatic hydrolysis in the outer range of the gradient. This led to lower MCHO and TCHO concentrations. Thus, the release of low molecular weight substrates by enzymatic degradation may be the limiting step in bacterial production.

In summary, we demonstrated that the concentrations of dissolved carbohydrates and carbohydrate enzyme activities did not show conservative behaviour in the salinity gradient. Their distribution is the result of multiple

biological interactions in combination with physical mixing processes. Concentrations of dissolved carbohydrates are influenced by the relationship between uptake and enzymatic release by bacteria and perhaps by the relationship between autotrophic and heterotrophic processes. Glucosidase activities in the gradient may be the result of the combination of substrate availability and the abundance of particle-associated bacteria with high hydrolytic potential.

Acknowledgements

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