Bacterial community structure influenced by *Coscinodiscus* sp. in the Vistula river plume*

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Abstract

The Gulf of Gdańsk is influenced by freshwater inflow from the River Vistula and by a wind-driven current along the coast. Bacterial communities from five stations along a salinity gradient were sampled during one day and analysed by terminal restriction fragment length polymorphism (T-RFLP), catalysed reporter deposition-fluorescence in situ hybridisation (CARD-FISH) and 16S rRNA gene libraries. On the day of sampling, we observed a probable current-driven seawater influx into the inner part of the gulf that separated the gulf into distinct water bodies. Members of the diatom *Coscinodiscus* sp. dominated one of these water bodies and influenced the bacterial community. The coexistence of typically freshwater and marine bacterioplankton populations in the Vistula river plume suggested an integration of some freshwater populations into the Baltic Sea bacterioplankton.

1. Introduction

Heterotrophic bacteria play a significant role in marine habitats. Causing organic matter to decay and mineralise, they are, together with the phytoplankton, the most important organisms responsible for the carbon cycle in fresh and marine waters (Hoppe et al. 2002). Intensively respiring bacteria influence the carbon dioxide concentration in the hydrosphere and indirectly in the atmosphere. Moreover, chemotrophic bacteria use dissolved organic matter (DOM) to build up fresh particulate matter. Thus, they play an important part in the carbon cycle, often called the microbial loop in the food web (Azam et al. 1983).

After the Black Sea, the Baltic Sea is the second largest brackish sea in the world. Its salinity ranges from 2 to 30. In the southern Baltic Proper and the Gulf of Gdańsk, the salinity of the surface layer oscillates around 7. Such conditions permit the real coexistence of marine and freshwater bacteria, as observed in the Baltic Sea (Riemann et al. 2008, Holmfeldt et al. 2009, Herlemann et al. 2011). The metabolic activity of freshwater bacteria and their importance in bacterial production was confirmed by Piwosz et al. (2013).

Compared to other Baltic Sea regions, the Gulf of Gdańsk is a highly productive region and the high level of community respiration makes the system net-heterotrophic (Witek et al. 1997). The River Vistula is the second largest river flowing into the Baltic Sea (mean annual flow rate – 1081 $m^3 s^{-1}$, HELCOM 2004). Since the end of 19th century, the Vistula has entered the Gulf of Gdańsk directly through an artificial channel. This direct inflow without a transitional estuary causes the water masses to mix

in the gulf. Depending on the wind and the currents, the two water bodies can mix vigorously, creating dynamic water fronts or broken off portions of riverine waters moving into the gulf as freshwater plumes. Conveyed by rivers, terrestrial organic matter may be a very important source of energy for the Baltic's trophic levels (Rolff & Elmgren 2000).

In recent years, the structure and activity of bacterial communities have been investigated in several estuaries along salinity gradients (Langenheder et al. 2004, Kirchman et al. 2005, Campbell & Kirchman 2013). In the Skagerrak-Kattegat water front area, along salinity gradients ranging from 21 to 30, differences in bacterioplankton composition were due to qualitative differences in bacterial growth conditions, as documented by changes in phytoplankton biomass, dissolved organic carbon and bacterial production (Pinhassi et al. 2003).

The Landsort Deep surface waters (salinity between 5.9 and 6.7) were dominated by *Bacteroidetes* and a mixture of typical freshwater bacteria like *Actinobacteria*, *Verrucomicrobia* and *Betaproteobacteria*. Marine taxa were not found (Riemann et al. 2008). In the coastal zone of the Gulf of Gdańsk (salinity ca 7), Piwosz et al. (2013) recorded the activity of freshwater lineages of acI *Actinobacteria*, LD12 *Alphaproteobacteria* and the betaproteobacterial genus *Limnohabitans* (R-BT), while the marine lineage SAR11 was thought to have originated from a passive inflow from the Baltic Proper. Studies performed along the 2000 km salinity gradient of the Baltic Sea showed that marine SAR11 and *Rhodobacteriaceae* were noted mainly in the marine part of the Baltic Sea or below 50 m depth in the Baltic Proper (Herlemann et al. 2011). *Roseobacter*, which are very abundant in marine environments and also culturable, have been broadly studied from different aspects (Buchan et al. 2005, Wagner-Döbler & Biebl 2006, Dang et al. 2008). They are often associated with diatoms in cultures (Allgaier et al. 2003) and frequently observed in the phytoplankton-attached fraction of bacterioplankton in environmental samples (Rooney-Varga et al. 2005).

In a previous study, a significantly higher bacterial production to primary production ratio was observed in the inner part of the Gulf of Gdańsk (Ameryk et al. 2005). The aim of this study was to investigate changes along the salinity gradient, as well as other environmental parameters, with the focus on the abundance and composition of bacterioplankton populations. Bacterial interactions with some phytoplankton organisms, especially *Coscinodiscus* sp. were noted by chance. Based on a wide range of methods, this study gave a precise snapshot of the microbial system observed during one sampling day. It is the first such detailed study of the bacterial composition in riverine transformed waters with salinities of ca 7.

2. Material and methods

2.1. Sampling site

The Gulf of Gdańsk is situated on the southern Baltic Sea coast. The time necessary for a complete water exchange with the open sea is about 15 days (Witek et al. 2003). The gulf is supplied by freshwater from the River Vistula, which slightly reduces its salinity in comparison to the Baltic Proper (6–7 vs. 7–8). The surface water samples were collected August 31, 2008 on the road bridge at Kiezmark over the Vistula (KIE) and also during a r/v 'Baltica' cruise at four different stations (ZN2, E53, E54, E62; Figure 1) along a salinity gradient ranging from 0.33 (river station KIE) to 7.25 (sea station E62). Conductivity, temperature and depth were measured using a CTD-rosette from on board the vessel.

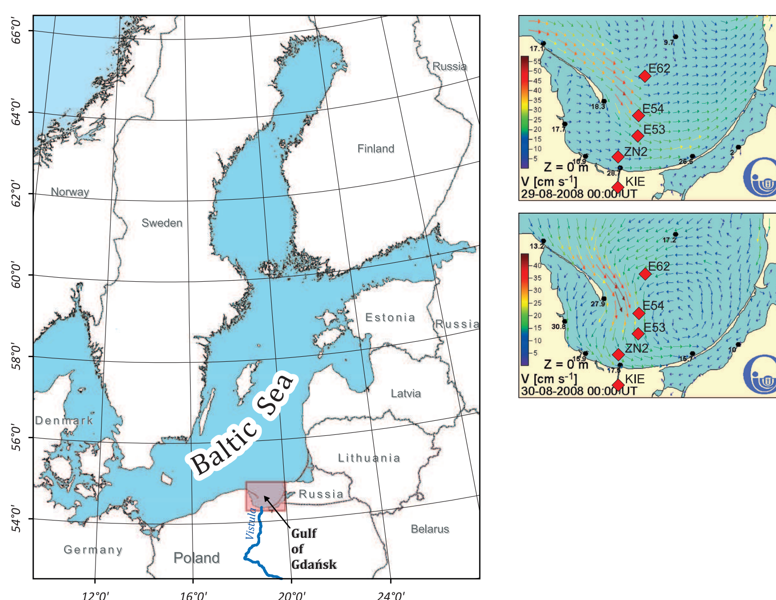

Figure 1. Sampling stations (KIE, ZN2, E53, E54, E62) shown against the background of the current situation one and two days before sampling (current maps from the University of Gdańsk ecohydrodynamic model http://model.ocean.ug.edu.pl/, Jędrasik et al. 2008, Kowalewski & Kowalewska-Kalkowska 2011). Black dots – numerical values of current velocities

2.2. Primary production, chlorophyll *a* and phytoplankton composition

Primary production was determined using the ^{14}C method (Evans et al. 1987, HELCOM 1988). For measurements of chlorophyll *a* and

phaeopigment concentrations, a fluorometric method with acetone extraction was used (Evans et al. 1987). The assimilation number (AN), which shows the efficiency of phytoplankton production, was calculated by dividing the primary production by the chlorophyll *a* concentration.

For the phytoplankton analysis, 200 ml of the surface water samples were immediately fixed with acidic Lugol's solution to a final concentration of 0.5% (Edler 1979). Subsamples of 20 ml were analysed using an inverted microscope Olympus IMT-2 with phase contrast and DIC. The individual phytoplankton cells were counted according to the Helsinki Commission recommendations (HELCOM 2001) and the biomass was calculated according to Olenina et al. (2006).

2.3. Dissolved organic carbon and nutrients

Samples for measuring the concentration of dissolved organic carbon (DOC) were stored in the dark at $-20°$C. Nitrocellulose filters (Millipore, 0.45 μm pore size) previously rinsed with deionised water were used for filtering the defrosted samples before analysis. DOC analyses were conducted by high-temperature combustion (HTC) (Shimadzu TOC-5000 analyser, Japan) (Dunalska et al. 2012). The quality of the dissolved organic matter was measured by using specific ultraviolet absorbance (SUVA), defined as the UV absorbance of a water sample at a given wavelength, normalised against DOC concentration. A spectrophotometer (Shimadzu UV-1601PC, Japan) was used to measure the UV absorbance (at 260 nm) in the water samples (Fukushima et al. 1996).

Nutrients such as nitrite, nitrate, ammonium, orthophosphate, silicates, total nitrogen and total phosphorus were freshly analysed on board, according to the recommendation of the Baltic Monitoring Programme (Grasshoff et al. 1983, UNESCO 1983, BMEPC 1988).

2.4. Bacterial numbers and biomass

Water samples were fixed with formaldehyde (final 1%), stained for 5 min with 4′,6-diamidino-2-phenylindole (DAPI, Sigma Aldrich, USA) (final 1 μg ml^{-1}), filtered on polycarbonate black membrane filters and stored at $-20°$C. The bacterial biomass (BBM) was determined after conducting cell measurements under an Olympus BX50 microscope (New Porton G12 eyepiece graticule). The biovolume of the cells was calculated using the following formula for a prolate spheroid: $V = (\pi/4)W^2(L - W/3)$ where $W =$ cell width and $L =$ cell length. A conversion factor of 0.35 pgC μm^{-3} (Bjørnsen 1986) was used to calculate the carbon biomass from the biovolume of the cells.

To obtain total bacterial cell counts (TCC), fixed samples (1% formaldehyde) were incubated for 30 min with 5 mM (final conc.) EDTA (for dissolving aggregates), stained for 15 min with SYBR Green (1x, Sigma Aldrich, USA) and analysed with a BDbiosciences FACS Calibur flow cytometer. The flow cytometer was equipped with an argon ion laser (15 mW) and the 488 nm emission line was used as the light source. Right-angle light scatter (SSC) was detected with a 488/10 nm band-pass filter and fluorescence (FL1) with a 530/30 nm band-pass filter. The system threshold was set to FL1 and SSC. The sample flow was calibrated by weighing three sets of water samples before and after each set of samples. The salinities (densities) of the samples were included in the calculations.

2.5. Catalysed Reporter Deposition-Fluorescence In Situ Hybridisation (CARD-FISH)

10 ml water samples were fixed with filtered formaldehyde (final conc. 1%), filtered on polycarbonate white filters (Osmonics INC., Poretics, 0.2 μm pore size, diameter 47 mm), rinsed with 100 ml sterile distilled water, dried and stored at -20°C. CARD FISH hybridisation was performed according to the protocol of Pernthaler et al. (2004). Oligonucleotide probes with horse-radish peroxidase were used to specifically stain bacterial populations (Table S1, see page 853). CARD-FISH preparations were evaluated on an epifluorescence microscope from Zeiss Axiophot. The photomicrographs were taken using an Axio Vision Camera (Carl Zeiss, Jena, Germany), and the bacteria were counted manually by ImageJ (Collins 2007). At least 1000 DAPI-stained cells per sample were counted. The nonEUB counts were non- or individual (1–2) cells per filter and therefore neglected. Relative numbers were based on DAPI counts. In the case of *Bacteria*, *Alphaproteobacteria*, *Betaproteobacteria* and *Actinobacteria*, the mean percentage of hybridised cells were calculated from two filters.

2.6. Bacterial production and activity

The bacterial biomass production was determined by the ^{3}H-leucine uptake method (Kirchman et al. 1985), using a mixture of radioactive leucine (8.3 nmol l^{-1}, specific activity 60 Ci mmol^{-1}) and non-radioactive leucine (100 nM) (Hoppe et al. 1998). Triplicates and a negative control (fixed with 1% formaldehyde, final concentration) were incubated at the in situ temperature for one hour. The incubation was stopped by adding sterile filtered formaldehyde (final conc. 1%). The protein production (BPP) was calculated based on the equation of Simon & Azam (1989), assuming an intracellular leucine isotope dilution of two. The cell-specific exponential

growth u was calculated with the equation $u = \ln((\mathrm{BBM} + \mathrm{BPP})/\mathrm{BBM})$. The doubling time (DT) was calculated with the equation $\mathrm{DT} = \ln(2)/u$ (Crump et al. 2004).

2.7. DNA extraction and T-RFLP analysis

Genomic DNA was isolated, following the protocol of Boström et al. (2004), from one half of a filter, representing 50 ml of water samples. The DNA was quantified with a NanoDrop ND-1000 Spectrophotometer (NanoDrop Technologies Inc., Wilmington, DE, USA) and yielded 10–50 ng genomic DNA per 100 ml water sample.

A terminal-restriction fragment length polymorphism (T-RFLP) analysis was performed following the protocol of Hahnke et al. (2013). In short: the fluorescently labelled general bacterial primers 27F (FAM, 5'-AGA GTT TGA TCC TGG CTC AG-3') and 907R (HEX, 5'-CCG TCA ATT CCT TTR AGT TT-3') were used to amplify the partial 16S rRNA gene (Muyzer et al. 1995). Approximately 25 ng of purified PCR products were digested with 5 U of the restriction enzyme *AluI*. The terminal restriction fragments (TRFs) were detected on an ABI Prism 3130 XL Genetic Analyzer (Applied Biosystems, California), equipped with an 80 cm capillary, a POP-7 polymer and the filter set D (Filter DS-30). The ROX-labelled MapMarker 1000 (Eurogentec, Belgium) served as a size standard between 50 bp and 1000 bp. Forward TRFs were analysed only because of the higher variability at the beginning of the 16S rRNA gene (Hahnke et al. 2013).

The T-RFLP patterns were analysed following the protocol of Hahnke et al. (2013). In short: TRFs between 50 and 1000 bp were identified and sized with the Genetic Analyser 3.7 (Applied Biosystems, California, USA) software, using a fluorescence intensity threshold of 20 U. The individual patterns were processed, applying the interactive binner (Ramette 2009) in R (http://www.r-project.org, version 2.3.1). The binning size was one nucleotide and the binning shift 0.1 nucleotides. The TRFs were named by subtracting 0.1 bases from the TRF length. The resulting pattern with normalised relative fluorescence intensities (RFI) were visualised in rank versus cumulated abundance curves with the k-dominance plot in PRIMER (v.6, PRIMER-E, Plymouth Marine Laboratory, UK) (Clarke 1993), in order to identify and remove outlying samples within the triplicates (one from station E53 and one from station E54) and identify the final T-RFLP data set. Fragments smaller than 100 nt were not included. There was a shift between closely situated intensive fluorescence peaks, which impaired data interpretation. Fragments of 230–232 nt were therefore excluded from analysis.

Visual comparisons between bacterial communities at each station were explored by ordination using non-metric multidimensional scaling (nMDS) and applying the isoMDS function of the MASS package (Venables & Ripley 2002) with 100 random restarts, Bray-Curtis dissimilarity and 999 iterations. The environmental parameters were fitted into the nMDS plot by applying the function envfit of the R package VEGAN (v.1.8–3, Dixon 2003) with 1000 permutations, Euclidian distances and P-values smaller than 0.001. The MANTEL test of the Vegan package in R was used to determine the Pearson product moment correlation of the TRF pattern at each station with environmental parameters. Similarity percentage analysis (SIMPER) and principal component analysis (PCA), overlain with Bray-Curtis similarity using PRIMER 6 (PRIMER Ltd., Plymouth, UK, Plymouth Marine Laboratory, UK) (Clarke 1993), were used to identify the TRFs that contributed most to the dissimilarity between stations.

2.8. Clone library construction

One microlitre of DNA extract from sample E54 was the template for the PCR reaction, using universal bacterial primers GM3 (5'-AGA GTT TGA TCC TGG C-3') and 1507R (5'-TAC CTT GTT ACG ACT T-3') for the 16S rRNA gene (Muyzer et al. 1995). The PCR reaction contained 25 μl PCR Master Mix (Promega GmbH, Mannheim, Germany) and 4 μM of forward and reverse primer in 50 μl. The cycle programme was 94°C for 1 min, 25 cycles of 94°C for 1 min, 42°C for 1 min, and 72°C for 3 min, followed by 60°C for 60 min. The PCR amplicons were purified on Sephadex columns (Sephadex^{TM} G-50 Superfine, Amersham Bioscience AB, Uppsala, Sweden) and approximately 10 ng DNA were cloned with a PCR 4.0-TOPO kit, following the manufacturer's instructions (Invitrogen, Carlsbad, CA, USA). Positive clones were selected by ampicillin resistance and the blue or white colony colour. The cloned and amplified 16S rRNA sequences were purified on Sephadex columns. The sequencing reaction was determined using the ABI Dye Terminator technology and the Applied Biosystems 3130xl DNAsequencer (Applied Biosystems, Foster City, USA). The 16S rRNA gene sequences were analysed with Sequencing Analysis 5.2 (Applied Biosystems, Foster City, USA) and assembled with Sequencer 4.6 (Gene codes, Ann Arbor, MI). Bellerophon (Huber et al. 2004), Chimera-Check (DeSantis et al. 2006), DECIPHER (Wright et al. 2012) and BLAST (Zhang et al. 2000) were used to check for chimeras. From each full length 16S rRNA gene sequence the primer sequences were removed. The initial phylogenetic affiliation was assigned using SeqMatch (Wang et al. 2007) of the Ribosomal Database Project (Cole et al. 2009).

Sequences were aligned with the SINA online aligner tool (www.arb-sina.de) (Pruesse et al. 2012). The alignment was imported into the ARB and manually corrected. Sequences were incorporated into the 16S rRNA tree (SILVA rel 111) by the parsimony method. Phylogenetic affiliation was assigned based on information in the tree. Clones of phytoplankton plasmids (15 of all 101 submitted clones) were excluded from further analyses. The 16S rRNA gene sequences were deposited under Acc. No. KF596513 – KF596613.

2.9. In silico prediction of fragment size

With Lasergene SeqBuilder (DNASTAR) the length of the in silico terminal restriction fragments (iTRF) of 16S rRNA gene sequences were determined by (i) trimming the sequences at the restriction recognition site of the restriction enzyme *AluI*, and (ii) adding the 20 nucleotides of the forward primer 27F to each sequence. The online programs MiCA 3 (http://mica.ibest.uidaho.edu, Shyu et al. 2007) and TRFragCalc (Hahnke et al. 2013) were used for in silico prediction of important TRFs absent from the clone library.

3. Results

3.1. Environmental factors measured at the sampling sites

The freshwater station in the River Vistula at Kiezmark (KIE) differed from the station in the vicinity of the river mouth – ZN2 and the seawater stations E53, E54 and E62 in that salinities and silicate concentrations were both lower (Table 1). The water temperature (17.3–18.9°C) was relatively constant at all stations. The large differences in salinity (between KIE and ZN2), together with the linear vertical salinity and temperature profiles (down to 20 m depth, data not shown), indicated a mixing of freshwater with the seawater in the river mouth or upstream of station ZN2.

The nutrient concentrations were in the micromolar range, but generally 2–25 times higher (except silicates) at the Kiezmark station (Table 1). At the same station, the concentration of dissolved organic carbon was the highest (5.6 mgC dm^{-3}), but simultaneously less labile. Allochthonous organic matter, as determined by the specific ultraviolet absorbance measurements (SUVA) (the higher the SUVA, the higher the ratio of molecules with aromatic rings and the less labile DOC), had its maximum at the river station KIE, with 18.8 dm^{-3} gC^{-1} cm^{-1} (Table 1). SUVA values (11.6–12.6 dm^3 gC^{-1} cm^{-1}) were the lowest at stations E53, E54 and E62, which potentially indicated DOC of phytoplankton origin.

Table 1. Measured chemical and biological parameters of the freshwater station KIE and the seawater stations ZN2, E53, E54, and E62

	Abbreviation	Unit	KIE	ZN2	E53	E54	E62
Temperature	Temp	°C	18.9	17.3	17.6	17.7	17.6
Salinity	Sal		0.3	7.1	7.1	7.0	7.2
Silicates	Si	μM	2.8	8.1	8.1	7.7	8.9
Total nitrogen	N_{tot}	μM	61.0	23.0	21.5	23.4	20.8
Nitrite	NO_2	μM	0.3	0.0	0.0	0.0	0.0
Nitrate	NO_3	μM	15.2	0.6	0.2	0.2	0.3
Ammonium	NH_4	μM	1.9	0.9	0.7	0.8	0.8
Organic nitrogen	N_{org}	μM	43.5	21.4	20.6	22.4	19.7
Total phosphorus	P_{tot}	μM	4.7	0.9	0.6	0.6	0.6
Orthophosphate	PO_4	μM	1.7	0.4	0.2	0.2	0.3
Organic phosphorus	P_{org}	μM	3.0	0.5	0.4	0.4	0.3
Chlorophyll *a*	Chl *a*	mg m^{-3}	63.5	4.0	3.1	3.2	3.1
Primary production	PP	mgC m^{-3} h^{-1}	331.6	22.8	17.7	22.0	21.2
Total bacterial cell counts	TCC	10^6 cm^{-3}	3.8	1.2	1.0	1.0	0.9
Bacterial protein production	BPP	mgC m^{-3} d^{-1}	34.0	7.3	6.1	6.2	3.2
Dissolved organic carbon	DOC	mgC dm^{-3}	5.6	4.3	4.5	4.3	4.5
Specific ultraviolet absorbance	SUVA	dm^3 gC^{-1} cm^{-1}	18.8	13.5	12.2	12.6	11.6

Interestingly, station E54 differed from the neighbouring stations E53 and E62 in terms of its organic nitrogen and silica concentrations. We suggest that the slightly higher organic nitrogen content and the reduced silica content indicated a local water body. According to the ecohydrodynamic model of the University of Gdańsk (http://model.ocean.ug.edu.pl/, Jędrasik et al. 2008, Kowalewski & Kowalewska-Kalkowska 2011), three days before sampling, a strong south-easterly current along the Hel Peninsula had pushed water masses from the open sea into the inner parts of the Gulf of Gdańsk (Figure 1). The more saline waters at stations ZN2 and E53 may have originated from the open sea, whereas the water around station E54 was a separate 'aged gulf' water body.

3.2. Phytoplankton communities and primary production

The freshwater Kiezmark station had the most productive phytoplankton community. The concentration of chlorophyll *a* (Table 1) coincided with the biomass of phytoplankton (Figure 2) and the highest primary production

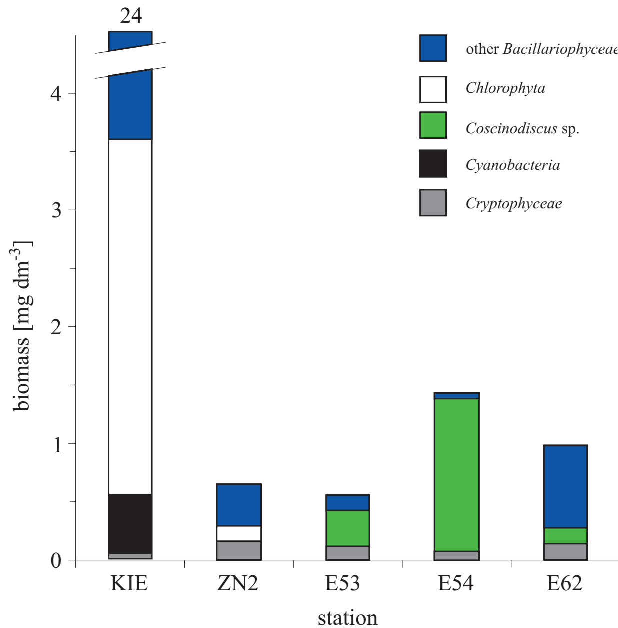

Figure 2. Biomass of phytoplankton groups present at the freshwater station KIE and the seawater stations ZN2, E53, E54 and E62

(Table 1). Our microscopic inspection detected 67 taxa, of which 32 belonged to green-algae, 10 to cyanobacteria and 8 to diatoms. Quantitatively, 85% of the phytoplankton biomass were diatoms. The dominant species was diatom *Cyclotella meneghiniana* (77% of the total phytoplankton biomass). Freshwater species were represented by *Skeletonema subsalsum* (2%) and the green-algae *Pediastrum duplex* (2%) and *Chlamydomonadales* (2%).

The highest growth efficiency of phytoplankton (assimilation number, AN) was found at the river mouth station ZN2 (Figure 3). This location reflects the direct influence of the River Vistula, where nutrient concentrations were higher compared to the other seawater stations. At the ZN2 sampling point, the highest taxonomic diversity in the phytoplankton community was observed. Among 73 taxa, 31 belonged to green-algae, 10 to diatoms and 8 to cyanobacteria. The dominance of the phytoplankton biomass by diatoms was noticeable at this station as well. They constituted 47% of the total phytoplankton biomass, including undefined *Centrales* 10–60 μm in diameter (36%), *Actinocyclus octonarius* var. *octonarius* (6%), *C. meneghiniana* (3%). *Cryptophyceae* constituted 22%, including *Teleaulax* spp. (15%) and *Plagioselmis prolonga* (7%), green-algae made up 18%, including the most frequent species *Pediastrum boryanum* (5%),

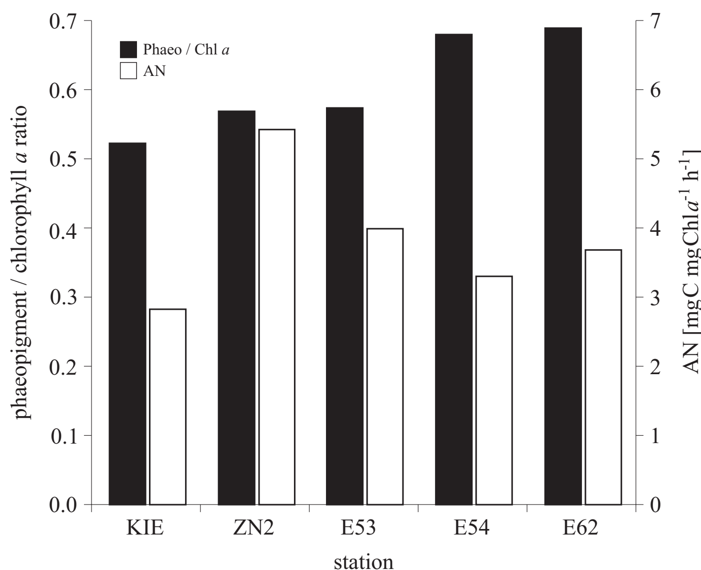

Figure 3. Phaeopigment/chlorophyll *a* ratio – left axis and assimilation number (AN) – right axis in the Gulf of Gdańsk

and dinoflagellates contributed 6%, including the most frequent genus *Protoperidinium* (5%).

Stations E54 and E62 had the highest proportion of decomposed chlorophyll *a* relative to intact chlorophyll *a* (phaeopigment/chlorophyll *a* ratio), which indicated accelerated phytoplankton decomposition (Figure 3). All the seawater stations (E53, E54 and E62) were similar in terms of phytoplankton diversity. The number of taxa was low (28–37), and the biomass was dominated by diatoms (63–90%) and *Cryptophyceae* (5–16%), while only a few cyanobacteria species were observed. The diatom *Coscinodiscus* sp. was the main component at station E54, constituting 88% of the whole phytoplankton biomass there. At stations E53 and E62 this diatom was less abundant (Figure 2); *A. octonarius* var. *octonarius* (4–57%), the *Cryptophyceae Teleaulax* spp. (11%) and *P. prolonga* (4–5%), as well as the ciliate *Mesodinium rubrum* (4%) contributed to the biomass of phytoplankton. The clone library (station E54) contained, besides bacterioplankton, some eukaryotic sequences, mostly of phytoplankton: 7 *Chlorophyta*, 6 *Stramenopiles*, 1 *Haptophyceae* and 1 *Alveolata*.

3.3. Genetic fingerprints of bacterioplankton communities and the environment

Terminal restriction fragment length polymorphism (T-RFLP) analysis based on the 16S rRNA gene diversity illustrated the differences in bacterial

communities among the sampling sites. Each terminal restriction fragment (TRF) represents an operational taxonomic unit (OTU). The presence of TRFs in a sample and their relative abundance are indications of differences between bacterial communities. Overall, 232 terminal restriction fragments (TRFs) were identified, with 52–95 TRFs (median 75 TRFs) per individual sample. We statistically analysed the presence and relative abundance of TRFs and investigated environmental parameters to gain further insights into the ecosystem.

The nMDS, CCA and PCA analyses suggested a separation of bacterioplankton communities into populations inhabiting the inner part of the gulf (E53, ZN2) and the outer part of the gulf together with the open sea (E54, E62) (Figure 5, see page 836). The Kiezmark station was excluded from the statistical analysis, because the biological and environmental parameters there had much higher values. CCA explained 77% of the variability (inertia of total variance = 1.3483, inertia of the first two constrained axes = 1.0441) and PCA 63.2% (34.6% PC axes 1, 28.6% PC axes 2). In the PCA analysis, the eigenvector of TRF_194nt and TRF_271nt pointed to samples

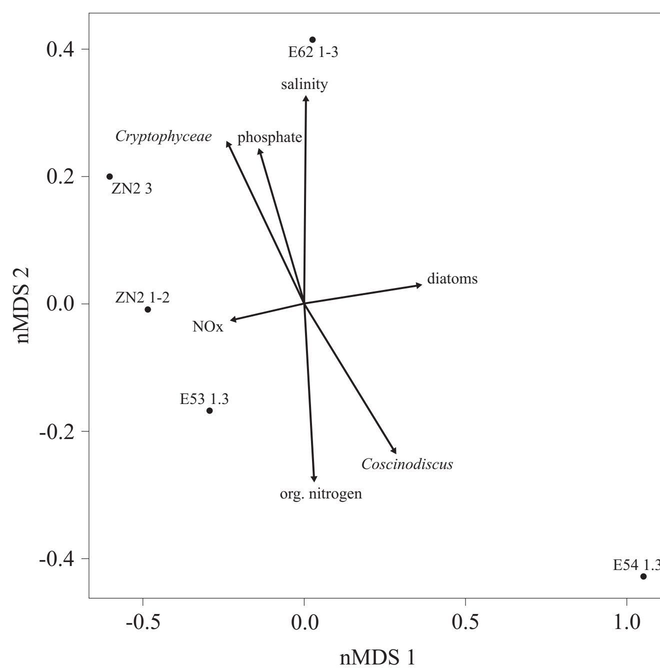

Figure 4. nMDS biplot of Bray-Curtis dissimilarities between stations ZN2, E53, E54, E62 (dots) based on TRF abundances with Pearson correlation of significant ($P < 0.001$, MANTEL) environmental and biological parameters (arrows). Stress, 0.02

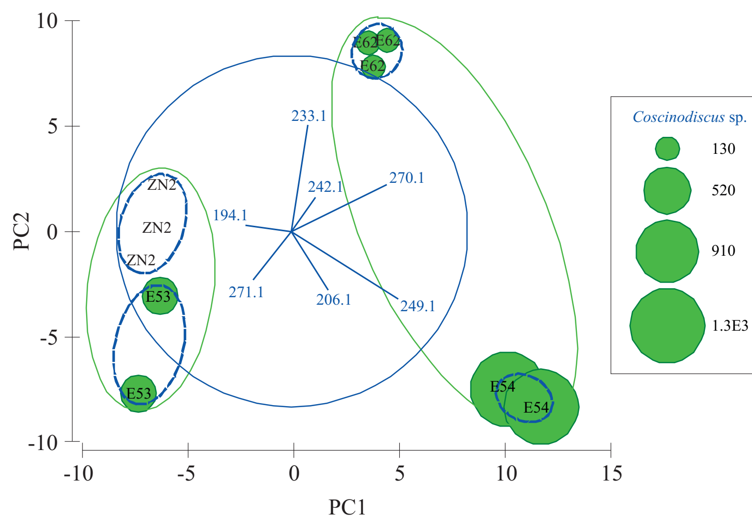

Figure 5. Principal component analyses (PCA) overlain with Bray-Curtis similarity of TRFs from stations (ZN2, E53, E54, E62) with the biomass of *Coscinodiscus* sp. shown as bubbles. Base variables correlation > 0.23. Green line 45% and blue dashed line 65% similarity

from the inner part of the gulf, whereas the eigenvectors of TRF_233nt, TRF_242nt, TRF_270nt, TRF_206nt and TRF_249nt pointed to samples from the outer part of the gulf and the open sea. TRF_249nt and TRF_206nt had the strongest influence on the discrimination of station E54 (the longest eigenvector in the direction of station E54).

Both the nMDS biplot of the Bray-Curtis dissimilarities between stations ZN2, E53, E54 and E62 based on TRF (Figure 4) and the principal component analysis (PCA) (Figure 5) detected a separation of station E54 (mean dissimilarity 61.5% SIMPER) from all the other stations. The correlation of environmental parameters with the bacterial community composition (MANTEL test) identified the biomass of *Coscinodiscus* sp. ($\rho = 0.78$, $P = 0.001$) and *Cryptophyceae* ($\rho = 0.79$, $P = 0.001$), the concentration of organic nitrogen ($\rho = 0.61$, $P = 0.002$) and salinity ($\rho = 0.60$, $P = 0.001$) as the most important independent factors explaining the separation of station E54 (Table S2, see page 854).

Individual TRFs were used to trace differences between bacterial communities in the water bodies using similarity percentage analysis (SIMPER, Table 2). The two fragments – TRF_274nt and TRF_242nt – were detected at all stations. The Kiezmark river station was characterised by TRF_140nt, TRF_195nt and TRF_161nt, accounting for 25.6% RFI. TRF_194nt was significant at the river mouth station ZN2. TRF_152nt,

Table 2. Abundance (relative fluorescence intensity, RFI) and significance of terminal restriction fragments (TRF) representative of the freshwater sampling site KIE, the mixed water sampling sites ZN2 and E53, the phytoplankton bloom sampling site E54, and the open sea sampling site E62. Listed are the average RFI at the sampling sites, the average RFI of TRFs at the sampling site (in) and at all other sampling sites (out), and mean dissimilarity (Diss/SD) from SIMPER. Additionally, the same information is given for the sum of TRF (Σ) representative of the sampling site

TRF [nt]		Average RFI at stations [%]							Diss/SD
		KIE	ZN2	E53	E54	E62	out	in	
KIE (freshwater)									
140		14.9	0.0	0.2	0.2	3.1	0.9	14.9	10.0
195		8.1	0.9	0.3	0.1	1.1	0.6	8.1	12.0
161		2.5	0.0	0.0	0.0	0.6	0.2	2.5	8.4
	Σ	25.6	0.9	0.5	0.3	4.8	1.8	25.6	12.4
ZN2 (mixed water)									
194		0.0	6.7	0.0	0.0	0.2	0.1	6.9	15.5
E53 (mixed water)									
272		0.9	1.2	15.3	0.3	2.2	1.2	15.3	19.0
152		0.4	0.3	1.4	0.2	0.0	0.3	1.4	6.7
189		0.5	0.2	2.3	0.0	0.3	0.3	2.4	6.1
	Σ	1.7	1.7	19.0	0.6	2.6	1.8	19.1	17.7
ZN2–E53									
542		0.0	3.8	3.0	0.5	0.0	0.2	2.7	1.9
241		0.9	1.3	2.1	0.2	1.1	0.7	1.6	1.6
147		0.1	1.0	1.0	0.0	0.0	0.0	1.0	17.0
	Σ	0.9	6.2	6.0	0.6	1.1	0.9	5.3	2.8
E54 (*Coscinodiscus* station)									
249		0.1	0.0	0.0	13.9	1.7	0.5	13.9	9.7
206		3.6	1.8	3.2	8.0	1.9	1.8	5.0	1.8
149		0.6	1.9	3.6	4.5	1.5	1.6	4.5	2.4
461		0.0	0.1	0.0	2.2	0.0	0.0	2.2	10.1
234		0.0	0.0	0.0	1.7	0.2	0.1	1.7	3.4
455		0.0	0.1	0.1	1.7	0.0	0.0	1.7	8.1
238		0.0	0.0	0.0	0.9	0.0	0.0	0.9	3.5
	Σ	4.4	3.8	6.9	32.9	5.3	3.9	29.9	7.9
E62 (seawater)									
145		0.2	0.1	0.0	0.2	1.2	0.1	1.2	8.0

Table 2. (*continued*)

TRF [nt]		Average RFI at stations [%]							Diss/SD
		KIE	ZN2	E53	E54	E62	out	in	
E54–E62									
187		0.0	0.1	0.0	2.3	1.5	0.0	1.4	2.0
270		7.4	6.9	8.7	16.4	17.1	7.7	16.7	6.6
	Σ	7.4	7.0	8.7	18.7	18.6	7.68	18.2	10.1
all									
274		2.5	4.8	5.1	4.3	3.3	n.d.	n.d.	n.d.
242		4.5	1.9	2.3	3.0	6.0	n.d.	n.d.	n.d.
	Σ	7.0	6.7	7.3	7.3	9.3	n.d.	n.d.	n.d.

TRF_189nt and TRF_272nt (together 19.1% RFI) were representative of station E53, located in the inner part of the gulf. Seven significant TRFs accounted for 29.9% RFI at sampling site E54, where the large-scale occurrence of *Coscinodiscus* sp. was recorded. At this station, TRF_249nt had the highest RFI of 13.9%. TRF_145nt occurred in the open sea waters at station E62. The analysis revealed a high percentage of RFI, due to TRF_147nt, TRF_241nt and TRF_542nt in the inner part of the Gulf of Gdańsk. In the outer part of the gulf (stations E54 and E63), TRF_187nt and TRF_270nt accounted for 18.2% RFI. Thus, the bacterioplankton community of station E54 differed markedly from those of the freshwater, the river mouth and the Gulf of Gdańsk.

3.4. Bacterioplankton diversity

Because of the unique T-RFLP pattern at station E54, a 16S rRNA gene library was generated from this station. Of the 86 good-quality bacterial sequences, 35% belonged to *Alphaproteobacteria*. Among these, 31% were affiliated with the brackish and marine SAR11 type. *Actinobacteria* represented 23%, *Bacteroidetes* 16%, *Gammaproteobacteria* 8%, *Betaproteobacteria* 6%, *Cyanobacteria* 6% and *Planctomycetes* 5%. One clone was sequenced from *Verrucomicrobia* and one from *Roseobacter* (Table S3, see page 855). The sequence of *Roseobacter* corresponded to iTRF_249nt (in silico TRF of 249 nt in length) which was a characteristic TRF at station E54. Other important TRFs at station E54 were iTRF_149nt belonging to *Spartobacteriaceae*, iTRF_461nt belonging to *Polaribacter* and iTRF_455nt belonging to *Flavobacterium*. Very likely, iTRF_140nt originated from the 16S rRNA gene sequence of *Flammeovirgaceae*, iTRF_233nt from *Actinobacterium* hgcl, and iTRF_270nt from *Verrucomicrobia*.

3.5. Bacterioplankton communities

Total bacterial cell counts (TCC) (1–3.8 cells 10^6 cm^{-3}) and bacterial protein production (BPP) (3.2–34 mgC m^{-3} d^{-1}) reached their maxima at the Kiezmark station (Table 1). The bacterial doubling times (DT) (19.8 h to 2.17 d) showed a reverse pattern (Figure 6). The doubling time of the investigated bacterioplankton was 20 hours in the river, 40 hours at station E54 and more than 2 days in the open sea. Bacterial biomass (BBM, 9.9–39.8 mgC m^{-3}) had the highest values in the river and decreased towards the open sea (Figure 6). Bacteria (EUBI-III) accounted for 38–69% of the total cell counts (DAPI). The amount of *Betaproteobacteria* and *Actinobacteria* (freshwater bacteria) was highest in the River Vistula (18.0% and 14.2%). In contrast, both bacterioplankton populations accounted for less than 5% of the total cell counts close to the river mouth, at station ZN2. With increasing distance from the land, the relative proportion of *Betaproteobacteria* and *Actinobacteria* decreased, and stayed constant at ca 3.5%, starting at station E53 and into the open Baltic Sea (Figure 7a). *Gammaproteobacteria* and *Roseobacter* achieved their maximum amounts (4.8% and 0.58%) at station E54. The SAR11 group was barely detectable, with a maximum amount (0.7%) at station E53 (Figure 7b). *Alphaproteobacteria* accounted for 5.7% of the total cell counts at the Kiezmark station and decreased to 2.2% at the open sea

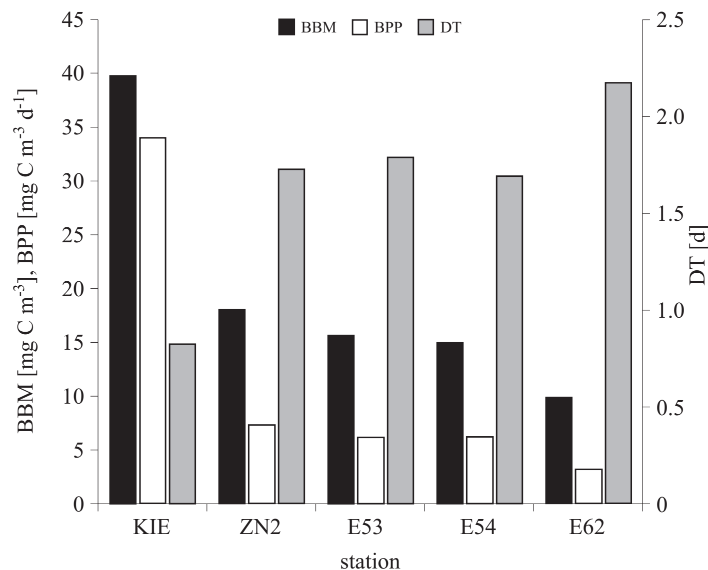

Figure 6. Bacterial biomass (BBM), bacterial protein production (BPP) and doubling time (DT)

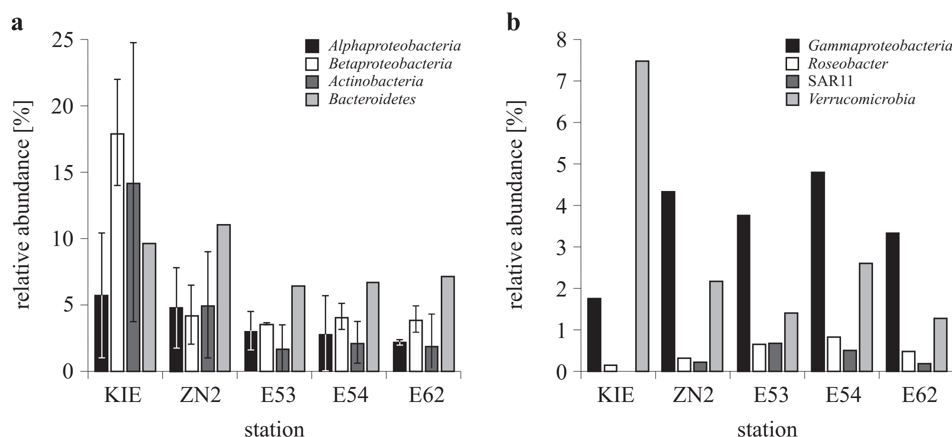

Figure 7. Percentage of abundance to total DAPI counts of (*Alphaproteobacteria*, *Betaproteobacteria*, *Gammaproteobacteria*, *Actinobacteria*) based on results from CARD-FISH. The error bars indicate standard deviations (a); CARD-FISH of less important bacterial groups (*Bacteroidetes*, *Roseobacter*, SAR11) (b) in the Gulf of Gdańsk

station E62. Members of the *Bacteroidetes* group accounted for 6.5%–11.1% (Figure 7b). The representative freshwater betaproteobacterium *Limnohabitans* was below the level of detection at all stations.

4. Discussion

In this study, we investigated the differences between the microbial communities of different water bodies in the Gulf of Gdańsk in late summer. The eutrophic waters of the Gulf of Gdańsk are phytoplankton-rich habitats during the growing season, lasting from April to October (Witek et al. 1997). The River Vistula stimulates both phytoplankton and bacterioplankton growth in the inner part of the Gulf of Gdańsk (Wielgat-Rychert et al. 2013). Allochthonous organic matter, as well as autochthonous matter of phytoplankton origin, are substrates which cause the growth of heterotrophic bacteria in the Gulf of Gdańsk (Ameryk et al. 2005). The phytoplankton composition in the Gulf of Gdańsk was typical for this season, as documented for the southern Baltic Proper since 2005 (Kownacka & Gromisz 2011). *Coscinodiscus* sp., which was the most important factor explaining the separation of station E54, is commonly present in the southern part of the Baltic Sea at the end of summer and in autumn (unpublished observation).

Members of the *Alphaproteobacteria*, *Betaproteobacteria*, *Gammaproteobacteria* and *Bacteroidetes* groups were repeatedly found associated with diatoms (Amin et al. 2012). In our study the three identified

TRFs (TRF_149nt, TRF_249nt and TRF_270nt) contributed significantly (35%) to the discrimination of station E54 from the other stations. Both TRF_149nt and TRF_270nt were affiliated with *Verrucomicrobia*, of which iTRF_149nt belonged to *Spartobacteriaceae* and iTRF_270nt to a 16S rRNA sequence of uncultured *Verrucomicrobia* (AM040118). The latter 16S rRNA sequence was found in the sediment off Sylt (Musat et al. 2006). Recently, *Verrucomicrobia* were observed in the Baltic Sea (Andersson et al. 2010) and *Spartobacteriaceae* were found to be quantitatively important in the Baltic Sea at salinities between 5 and 10 (Herlemann et al. 2011). *Verrucomicrobia*, which can make a considerable contribution to polysaccharide degradation, can also be expected to be associated with phytoplankton (Martinez-Garcia et al. 2012). *Spartobacteria* in particular have been directly associated with phytoplankton in the Baltic Sea (Herlemann et al. 2013).

TRF_249nt was identified as a candidate for *Roseobacter*. A clone sequence with this TRF was affiliated with the *Roseobacter* DC5-80-3 branch in the RCA cluster and CARD-FISH showed an abundance of less than 1%. The RCA cluster is widespread in temperate and polar oceans, but constituted less than 0.5% of all bacteria in the Baltic Sea (Selje et al. 2004). In surface waters, no representative was found at the Landsort Deep station (Riemann et al. 2008) or in the Baltic Proper (Herlemann et al. 2011). As its absence was observed in spring (Riemann et al. 2008) and summer (Herlemann et al. 2011) and its presence in late summer (our data) and in autumn (Selje et al. 2004), such differences may be explained by the seasonal dynamics of taxa within the Baltic Sea bacterioplankton communities (Andersson et al. 2010). *Roseobacter* was often shown to co-occur with phytoplankton (Buchan et al. 2005), especially with natural phytoplankton blooms (O'Sullivan et al. 2004) or in mesocosm studies of *Thalassiosira* (Allgaier et al. 2003). It was also shown to be an early surface coloniser in temperate marine waters (Dang et al. 2008); the DC5-80-3 clade has been linked with the degradation of aromatic compounds (Buchan et al. 2005).

Crump et al. (2004) showed that a shift from a mixture of allochthonous communities to a native estuarine community requires bacterial doubling times much shorter than the local water residence time. The doubling time (DT) calculated on the basis of leucine bacterial production and bacterial biomass (all DAPI stained cells) was about 1.7–2.2 days in the Gulf of Gdańsk; a shorter doubling time would probably be based on active cells only. The DT was at least seven times shorter than the residence time in the Gulf of Gdańsk, calculated by Witek et al. (2003). Bacteria in the water at station E54 had enough time to establish a stable community connected with the occurrence of *Coscinodiscus* sp.

Along increasing salinity gradients in estuaries, *Alphaproteobacteria* and *Gammaproteobacteria* populations increase, whereas *Betaproteobacteria* decrease, as shown for the bacterial populations in the Gulf of Delaware (Cottrell & Kirchman 2003), in Chesapeake Bay (Bouvier & del Giorgio 2002) and in the estuary of the River Pearl (Zhang et al. 2006). Our sampling data did not strictly follow a salinity gradient, but rather the distance from the river mouth, owing to the unexpected hydrological situation. However, the 16S rRNA gene library (station E54) revealed bacterial genera affiliated with marine, fresh and brackish waters.

Surprisingly, *Alphaproteobacteria* did not follow the expected pattern. In addition to the marine and brackish types, *Alphaproteobacteria* have a typically freshwater group, like the LD12 clade (the sister clade of SAR11). This group was recorded by Piwosz et al. (2013) in the Gulf of Gdańsk. The high amount of *Alphaproteobacteria* in Vistula waters might have been caused by a LD12 group characterised by a relatively small cell size. SAR11 itself had the highest number (27/86) of representatives in the clone library. Twenty-five of its clones belonged to the brackish clade of Chesapeake – Delaware Bay, and two to the oceanic clade surface 1. However, their relative abundance ratio did not exceed 0.7% and they were rather a minor fraction in the Gulf of Gdańsk bacterial community. SAR11 activity was investigated during different seasons in the coastal region of the Gulf of Gdańsk and showed low activity, which is probably due to the passive inflow of more saline waters from the Baltic Proper (Piwosz et al. 2013).

The marine *Bacteroides* (*Cytophagia*, *Flavobacteriia* and *Sphingobacteriia*) dominated the bacterioplankton community in the Landsort Deep (Riemann et al. 2008) and in the Gulf of Gdańsk. Five clone sequences were affiliated with *Sphingobacteriales* and eight with *Flavobacteriales*. The fresh-brackish clade *Fluviicola* (1 clone) was present, as well as the marine brackish clades NS3 and NS9, and *Owenweeksia* (1 clone each).

Actinobacteria, which are usually rare in pelagic marine systems (Pommier et al. 2007), were found to have significant autochthonic populations in the central Baltic Sea (Riemann et al. 2008). *Actinobacteria* accounted for 25% of the bacterioplankton in the Gulf of Bothnia (salinity 0–5) (Holmfeldt et al. 2009). The freshwater lineage acI was mainly active when the salinity in the Gulf of Gdańsk was low (Piwosz et al. 2013). Salinity changes may cause sudden changes in the amounts of *Actinobacteria* and *Betaproteobacteria*. Only *Verrucomicrobia*, the freshwater *Actinobacteria* lineage hgcl, and probably *Synechococcus* (TRF_194nt) were dominant in these waters. Many other groups (74 TRFs) accounted for less than 5% of all the bacterioplankton combined. Seven of the 20 *Actinobacteria*

clones were from the fresh-brackish clade hgcl and eleven from the marine *Acidimicrobiaceae* group.

Betaproteobacteria, which are mainly freshwater species (Zwart et al. 2002), were present in the Vistula as well as in the Gulf of Gdańsk. *Betaproteobacteria* were present at station E54, affiliating with the freshwater *Alcaligenaceae* MWH-UniP1, the coastal clade OM43 and the clade *Comamonadaceae* BAL58, which was isolated from the Baltic Proper (Simu & Hagström 2004). The bacterial community structure in the Baltic Sea is characterised by a large seasonal diversity change (Andersson et al. 2010). The lack of the freshwater betaproteobacterium *Limnohabitans* in August may be explained by its seasonal appearance just after the spring phytoplankton bloom in the Gulf of Gdańsk (Piwosz et al. 2013).

T-RFLP and the clone library, which are methods based on polymerase chain reactions, cannot be treated quantitatively. In contrast, CARD-FISH enables the counting of single cells and the comparison of relative abundances of the investigated bacterial groups. However, as there is no perfect oligonucleotide probe that targets only the group of interest, the use of probes that target broader bacterial groups at the phyla level carries the danger of over- or underestimation (Amann & Fuchs 2008). Relative bacterial numbers based on the CARD-FISH probes used in this study showed only a general picture of the community composition in the Vistula river plume.

5. Conclusions

The occurrence of the diatom *Coscinodiscus* sp. influenced the bacterial communities in the Gulf of Gdańsk. The mix of freshwater and typical marine bacteria exhibited a high diversity in this region. The change in environmental conditions from the river to the open sea may have caused the death of some freshwater bacteria, but some of them probably adapted to marine conditions and became an integral part of the southern Baltic Sea bacterioplankton.

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References

Allgaier M., Uphoff H., Felske A., Wagner-Döbler I., 2003, *Aerobic anoxygenic photosynthesis in Roseobacter clade bacteria from diverse marine habitats*, Appl. Environ. Microb., 69 (9), 5051–5059, http://dx.doi.org/10.1128/AEM.69.9.5051-5059.2003.

Amann R. I., Binder B. J., Olson R. J., Chisholm S. W., Devereux R., Stahl D. A., 1990, *Combination of 16S rRNA-targeted oligonucleotide probes with flow cytometry for analyzing mixed microbial populations*, Appl. Environ. Microb., 56, 1919–1925.

Amann R., Fuchs B. M., 2008, *Single-cell identification in microbial communities by improved fluorescence in situ hybridization techniques*, Nature, 6, 339–348.

Ameryk A., Podgórska B., Witek Z., 2005, *The dependence between bacterial production and environmental conditions in the Gulf of Gdańsk*, Oceanologia, 47 (1), 27–45.

Amin S. A., Parker M. S., Armbrust E. V., 2012, *Interactions between diatoms and bacteria*, Microbiol. Mol. Biol. Rev., 76 (3), 667–684, http://dx.doi.org/10.1128/MMBR.00007-12.

Andersson A. F., Riemann L., Bertilsson S., 2010, *Pyrosequencing reveals contrasting seasonal dynamics of taxa within Baltic Sea bacterioplankton communities*, ISME J., 4, 171–181, http://dx.doi.org/10.1038/ismej.2009.108.

Azam F., Fenchel T., Field J. G., Gray J. S., Meyer-Reil L. A., Thingstad F., 1983, *The ecological role of water-column microbes in the Sea*, Mar. Ecol.-Prog. Ser., 10, 257–263, http://dx.doi.org/10.3354/meps010257.

Björnsen P. K., 1986, *Automatic determination of bacterioplankton biomass by image analysis*, Appl. Environ. Microb., 51 (6), 1199–1204.

BMEPC, 1988, *Guidelines for the Baltic Monitoring Programme for the third stage*, Balt. Mar. Environ. Prot. Comm., Helsinki, 1–161.

Boström K. H., Simu K., Hagström Å., Riemann L., 2004, *Optimization of DNA extraction for quantitative marine bacterioplankton community analysis*, Limnol. Oceanogr.-Meth., 2, 365–373, http://dx.doi.org/10.4319/lom.2004.2.365.

Bouvier T. C., del Giorgio P. A., 2002, *Compositional changes in free-living bacterial communities along a salinity gradient in two temperate estuaries*, Limnol. Oceanogr., 47 (2), 453–470, http://dx.doi.org/10.4319/lo.2002.47.2.0453.

Buchan A., González J. M., Moran M. A., 2005, *Overview of the marine Roseobacter lineage*, Appl. Environ. Microb., 71 (10), 5665–5677, http://dx.doi.org/10.1128/AEM.71.10.5665-5677.2005.

Campbell B. J., Kirchman D. L., 2013, *Bacterial diversity, community structure and potential growth rates along an estuarine salinity gradient*, ISME J., 7, 210–220, http://dx.doi.org/10.1038/ismej.2012.93.

Clarke K. R., 1993, *Non-parametric multivariate analyses of changes in community structure*, Aust. J. Ecol., 18 (1), 117–143, http://dx.doi.org/10.1111/j.1442-9993.1993.tb00438.x.

Cole J. R., Wang Q., Cardenas E., Fish J., Chai B., Farris R. J., Kulam-Syed-Mohideen A. S., McGarrell D. M., Marsh T., Garrity G. M., Tiedje J. M., 2009, *The Ribosomal Database Project: improved alignments and new tools for rRNA analysis*, Nucleic Acids Res., 37 (S1), D141–D145, http://dx.doi.org/10.1093/nar/gkn879.

Collins T. J., 2007, *ImageJ for microscopy*, BioTechniques, 43 (Suppl. 1), 25–30.

Cottrell M. T., Kirchman D. L., 2003, *Contribution of major bacterial groups to bacterial biomass production (thymidine and leucine incorporation) in the Delaware estuary*, Limnol. Oceanogr., 48 (1), 168–178, http://dx.doi.org/10.4319/lo.2003.48.1.0168.

Crump B. C., Hopkinson C. S., Sogin M. L., Hobbie J. E., 2004, *Microbial biogeography along an estuarine salinity gradient: combined influences of bacterial growth and residence time*, Appl. Environ. Microb., 70 (3), 1494–1505, http://dx.doi.org/10.1128/AEM.70.3.1494-1505.2004.

Daims H., Brühl A., Amann R., Schleifer K.-H., Wagner M., 1999, *The domain-specific probe EUB338 is insufficient for the detection of all Bacteria: development and evaluation of a more comprehensive probe set*, Syst. Appl. Microbiol., 22 (2), 434–444, http://dx.doi.org/10.1016/S0723-2020(99)80053-8.

Dang H., Li T., Chen M., Huang G., 2008, *Cross-ocean distribution of Rhodobacterales bacteria as primary surface colonizers in temperature coastal marine waters*, Appl. Environ. Microb., 74 (1), 52–60, http://dx.doi.org/10.1128/AEM.01400-07.

DeSantis T. Z., Hugenholtz P., Larsen N., Rojas M., Brodie E. L., Keller K., Huber T., Dalevi D., Hu P., Andersen G. L., 2006, *Greengenes, a chimera-checked 16S rRNA gene database and workbench compatible with ARB*, Appl. Environ. Microb., 72 (7), 5069–5072, http://dx.doi.org/10.1128/AEM.03006-05.

Dixon P., 2003, *VEGAN, a package of R functions for community ecology*, J. Veg. Sci., 14 (6), 927–930, http://dx.doi.org/10.1111/j.1654-1103.2003.tb02228.x.

Dunalska J. A., Górniak D., Jaworska B., Evelyn E., Gaiser E. E., 2012, *Effect of temperature on organic matter transformation in a different ambient nutrient availability*, Ecol. Eng., 49, 27–34, http://dx.doi.org/10.1016/j.ecoleng.2012.08.023.

Edler L., 1979, *Recommendations on methods for marine biological studies in the Baltic Sea. Phytoplankton and chlorophyll*, Balt. Mar. Biol. Publ., 5, 1–38.

Eilers H., Pernthaler J., Peplies J., Glöckner F. O., Gerdts G., Amann R., 2001, *Isolation of novel pelagic bacteria from the German Bight and their seasonal*

contributions to surface picoplankton, Appl. Environ. Microb., 67 (11), 5134 –5142, http://dx.doi.org/10.1128/AEM.67.11.5134-5142.2001.

Evans C. A., O'Reilly J. E., Thomas J. P., 1987, *A handbook for measurement of chlorophyll a and primary productivity*, BIOMASS Sci., 8, 114 pp.

Fukushima T., Park J., Imai A., Matsushige K., 1996, *Dissolved organic carbon in a eutrophic lake; dynamics, biodegradability and origin*, Aquat. Sci., 58 (2), 139–157, http://dx.doi.org/10.1007/BF00877112.

Grasshoff K., Erhardt M., Kremling K. (eds.), 1983, *Methods of sea water analysis*, Verlag Chemie, Weinheim, 63–97, 127–187.

Hahnke R. L., Probian C., Fuchs B. M., Harder J., 2013, *Variations of pelagic bacterial communities in the North Atlantic Ocean coincide with water bodies*, Aquat. Microbiol. Ecol., 71 (2), 131–140, http://dx.doi.org/10.3354/ame01668.

HELCOM, 1988, *Guidelines for the Baltic Monitoring Programme for the third stage: Part D, Biological determinands*, Balt. Sea Environ. Proc., 27 D, 1 –161.

HELCOM, 2001, *Manual for marine monitoring in the COMBINE programme of HELCOM. Part C. Programme for monitoring of eutrophication and its effects. Annex C-6: Phytoplankton species composition, abundance and biovolume*, Balt. Mar. Environ. Prot. Comm., Helsinki, http://www.helcom.fi/groups/monas/CombineManual/AnnexesC/en_GB/annex6/.

HELCOM, 2004, *The fourth Baltic Sea pollution load compilation (PLC-4)*, Balt. Sea Environ. Proc., 93, 188 pp.

Herlemann D. P. R., Labrenz M., Jürgens K., Bertilsson S., Waniek J. J., Andersson A. F., 2011, *Transitions in bacterial communities along the 2000 km salinity gradient of the Baltic Sea*, ISME J., 5, 1571–1579, http://dx.doi.org/10.1038/ismej.2011.41.

Herlemann D. P. R., Lundin D., Labrenz M., Jürgens K., Zheng Z., Aspeborg H., Andersson A. F., 2013, *Metagenomic de novo assembly of an aquatic representative of the Verrucomicrobial class Spartobacteria*, mBio, 4 (3), http://dx.doi.org/10.1128/mBio.00569-12.

Holmfeldt K., Dziallas C., Titelman J., Pohlmann K., Grossart H.-P., Riemann L., 2009, *Diversity and abundance of freshwater Actinobacteria along environmental gradients in the brackish northern Baltic Sea*, Environ. Microbiol., 11 (8), 2042–2054, http://dx.doi.org/10.1111/j.1462-2920.2009.01925.x.

Hoppe H.-G., Giesenhagen H. C., Gocke K., 1998, *Changing patterns of bacterial substrate decomposition in a eutrophication gradient*, Aquat. Microb. Ecol., 15 (1), 1–13, http://dx.doi.org/10.3354/ame015001.

Hoppe H-G., Gocke K., Koppe R., Begler C., 2002, *Bacterial growth and primary production along a north-south transect of the Atlantic Ocean*, Nature, 416, 168–171, http://dx.doi.org/10.1038/416168a.

Huber T., Faulkner G., Hugenholtz P., 2004, *Bellerophon: a program to detect chimeric sequences in multiple sequence alignments*, Bioinformatics, 20 (14), 2317–2319, http://dx.doi.org/10.1093/bioinformatics/bth226.

Jędrasik J., Cieślikiewicz W., Kowalewski M., Bradtke K., Jankowski A., 2008, *44 years hindcast of the sea level and circulation in the Baltic Sea*, Coast. Eng., 55 (11), 849–860, http://dx.doi.org/10.1016/j.coastaleng.2008.02.026.

Kirchman D., K'nees E., Hodson R., 1985, *Leucine incorporation and its potential as a measure of protein synthesis by bacteria in natural aquatic systems*, Appl. Environ. Microb., 49 (3), 599–607.

Kirchman D. L., Dittel A. I., Malmstrom R. R., Cottrell M. T., 2005, *Biogeography of major bacterial groups in the Delaware Estuary*, Limnol. Oceanogr., 50 (5), 1697–1706, http://dx.doi.org/10.4319/lo.2005.50.5.1697.

Kowalewski M., Kowalewska-Kalkowska H., 2011, *Performance of operationally calculated hydrodynamic forecasts during storm surges in the Pomeranian Bay and Szczecin Lagoon*, Boreal Env. Res., 16 (Suppl. A), 27–41.

Kownacka J., Gromisz S., 2011, *Semi-decade dynamic of the summer phytoplankton community from the southern Baltic Proper*, ICES Annual Science Conferences, 19–23 September, Gdańsk, poster.

Langenheder S., Kisand V., Lindstrom E. S., Wikner J., Tranvik L. J., 2004, *Growth dynamics within bacterial communities in riverine and estuarine batch cultures*, Aquat. Microb. Ecol., 37 (2), 137–148, http://dx.doi.org/10.3354/ame037137.

Manz W., Amann R., Ludwig W., Wagner M., Schleifer K.-H., 1992, *Phylogenetic oligodeoxynucleotide probes for the major subclasses of Proteobacteria: problems and solutions*, Syst. Appl. Microbiol., 15 (4), 593–600, http://dx.doi.org/10.1016/S0723-2020(11)80121-9.

Manz W., Amann R., Ludwig W., Vancanneyt M., Schleifer K.-H., 1996, *Application of a suite of 16S rRNA-specific oligonucleotide probes designed to investigate bacteria of the phylum cytophaga-flavobacter-bacteroides in the natural environment*, Microbiology, 142 (5), 1097–1106, http://dx.doi.org/10.1099/13500872-142-5-1097.

Martinez-Garcia M., Brazel D. M., Swan B. K., Arnosti C., Chain P. S. G., Reitenga K. G., Xie G., Poulton N. J., Gomez M. L., Masland D. E. D., Thompson B., Bellows W. K., Ziervogel K., Lo C-C., Ahmed S., Gleasner C. D., Detter C. J., Stepanauskas R., 2012, *Capturing single cell genomes of active polysaccharide degraders: an unexpected contribution of Verrucomicrobia*, PLoS ONE, 7 (4), e35314, http://dx.doi.org/10.1371/journal.pone.0035314.

Morris R. M., Rappe M. S., Connon S. A., Vergin K. L., Siebold W. A., Carlson C. A., Giovannoni S. J., 2002, *SAR11 clade dominates ocean surface bacterioplankton communities*, Nature, 420, 806–810, http://dx.doi.org/10.1038/nature01240.

Musat N., Werner U., Knittel K., Kolb S., Dodenhof T., van Beusekom J. E., de Beer D., Dubilier N., Amann R., 2006, *Microbial community structure of sandy intertidal sediments in the North Sea, Sylt-Rømø Basin, Wadden Sea*, Syst.

Appl. Microbiol., 29 (4), 333–348, http://dx.doi.org/10.1016/j.syapm.2005.12.006.

Muyzer G., Teske A., Wirsen C., Jannasch H., 1995, *Phylogenetic relationships of Thiomicrospira species and their identification in deep-sea hydrothermal vent samples by denaturing gradient gel electrophoresis of 16S rDNA fragments*, Arch. Microbiol., 164 (3), 165–172, http://dx.doi.org/10.1007/BF02529967.

Neef A., 1997, *Anwendung der in situ Einzelzell-Identifizierung von Bakterien zur Populationsanalyse in komplexen mikrobiellen Biozönosen*, Ph. D. thesis, Tech. Univ., München.

Olenina I., Hajdu S., Edler L., Andersson A., Wasmund N., Busch S., Gobel J., Gromisz S., Huseby S., Huttunen M., Jaanus A., Kekkonen P., Ledaine I., Ziemkiewicz E., 2006, *Biovolumes and size-classes of phytoplankton in the Baltic Sea*, HELCOM Baltic Sea Environ. Proc., 106, 144 pp.

O'Sullivan L. A., Fuller K. E., Thomas E. M., Turley C. M., Fry J. C., Weightman A. J., 2004, *Distribution and culturability of the uncultivated 'AGG58 cluster' of the Bacteroidetes phylum in aquatic environments*, FEMS Microbiol. Ecol., 47 (3), 359–370, http://dx.doi.org/10.1016/S0168-6496(03)00300-3.

Pernthaler A., Pernthaler J., Amman R., 2004, *Sensitive multicolor fluorescence in situ hybridization for the identification of environmental microorganisms*, [in:] *Molecular microbial ecology manual*, 2nd edn., G. Kowalchuk, F. J. de Bruijn, I. M. Head, A. D. L. Akkermans & J. van Elsas (eds.), Kluwer Acad. Publ., Dordrecht, 711–126.

Pinhassi J., Winding A., Binnerup S. J., Zweifel U. L., Riemann B., Hagström Å., 2003, *Spatial variability in bacterioplankton community composition at the Skagerrak-Kattegat Front*, Mar. Ecol.-Prog. Ser., 255, 1–13, http://dx.doi.org/10.3354/meps255001.

Piwosz K., Salcher M. M., Zeder M., Ameryk A., Pernthaler J., 2013, *Seasonal population dynamics and activity of freshwater bacteria in brackish waters of the Gulf of Gdańsk*, Limnol. Oceanogr., 58 (3), 817–826.

Pommier T., Canbäck B., Riemann L., Boström K. H., Simu K., Lundberg P., Tunlid A., Hagström Å., 2007, *Global patterns of diversity and community structure in marine bacterioplankton*, Mol. Ecol., 16 (4), 867–880, http://dx.doi.org/10.1111/j.1365-294X.2006.03189.x.

Pruesse E., Peplies J., Glöckner F. O., 2012, *SINA: accurate high-throughput multiple sequence alignment of ribosomal RNA genes*, Bioinformatics, 28 (14), 1823–1829, http://dx.doi.org/10.1093/bioinformatics/bts252.

Ramette A., 2009, *Quantitative community Fingerprinting methods for estimating the abundance of operational taxonomic unit in natural microbial communities*, Appl. Environ. Microb., 75 (8), 2495–2505, http://dx.doi.org/10.1128/AEM.02409-08.

Riemann L., Leitet C., Pommier T., Simu K., Holmfeldt K., Larsson U., Hagström Å., 2008, *The native bacterioplankton community in the central Baltic Sea is influenced by freshwater bacterial species*, Appl. Environ. Microb., 74 (2), 503–515.

Rolff C., Elmgren R., 2000, *Use of riverine organic matter in plankton food webs of the Baltic Sea*, Mar. Ecol.-Prog. Ser., 197, 81–101, http://dx.doi.org/10.3354/meps197081.

Roller C., Wagner M., Amann R., Ludwig W., Schleifer K.-H., 1994, *In situ probing of Gram-positive bacteria with high DNA G+C content using 23S rRNA-targeted oligonucleotides*, Microbiology, 140, 2849–2858, http://dx.doi.org/10.1099/00221287-140-10-2849.

Rooney-Varga J. N., Giewat M. W., Savin M. C., Sood S., LeGresley M., Martin J. L., 2005, *Links between phytoplankton and bacterial community dynamics in a coastal marine environment*, Microb. Ecol., 49 (1), 163–175, http://dx.doi.org/10.1007/s00248-003-1057-0.

Selje N., Simon M., Brinkhoff T., 2004, *A newly discovered Roseobacter cluster in temperate and polar oceans*, Nature, 427, 445–448, http://dx.doi.org/10.1038/nature02272.

Shyu C., Soule T., Bent S. J., Foster J. A., Forney L. J., 2007, *MiCA: A web-based tool for the analysis of microbial communities based on terminal-restriction fragment length polymorphisms of 16S and 18S rRNA genes*, Microb. Ecol., 53 (4), 562–570, http://dx.doi.org/10.1007/s00248-006-9106-0.

Simon M., Azam F., 1989, *Protein content and protein synthesis rates of planktonic marine bacteria*, Mar. Ecol.-Prog. Ser., 51, 201–213, http://dx.doi.org/10.3354/meps051201.

Simu K., Hagström Å., 2004, *Oligotrophic bacterioplankton with a novel single-cell life strategy*, Appl. Environ. Microb., 70 (4), 2445–2451, http://dx.doi.org/10.1128/AEM.70.4.2445-2451.2004.

Šimek K., Pernthaler J., Weinbauer M. G., Hornak K., Dolan J. R., Nedoma J., Masin M., Amann R., 2001, *Changes in bacterial community composition and dynamics and viral mortality rates associated with enhanced flagellate grazing in a mesoeutrophic reservoir*, Appl. Environ. Microb., 67 (6), 2723–2733, http://dx.doi.org/10.1128/AEM.67.6.2723-2733.2001.

UNESCO, 1983, *Chemical methods for use in marine environmental monitoring. Manual and guides*, Intergovern. Oceanogr. Comm., 12, 1–53.

Venables W. N., Ripley B. D., 2002, *Modern applied statistics with S*, Springer, New York, http://dx.doi.org/10.1007/978-0-387-21706-2.

Wagner-Döbler I., Biebl H., 2006, *Environmental biology of the marine Roseobacter lineage*, Ann. Rev. Microbiol., 60, 255–280, http://dx.doi.org/10.1146/annurev.micro.60.080805.142115.

Wallner G., Amann R., Beisker W., 1993, *Optimizing fluorescent in situ hybridization with rRNA-targeted oligonucleotide probes for flow cytometric identification of microorganisms*, Cytometry, 14, 136–143.

Wang Q., Garrity G. M., Tiedje J. M., Cole J. R., 2007, *Naive Bayesian classifier for rapid assignment of rRNA sequences into the new bacterial taxonomy*, Appl. Environ. Microb., 73 (16), 5261–5267, http://dx.doi.org/10.1128/AEM.00062-07.

Wielgat-Rychert M., Ameryk A., Jarosiewicz A., Kownacka J., Rychert K., Szymanek L., Zalewski M., Agatova A., Lapina N., Torgunova N., 2013, *Impact of the inflow of Vistula river waters on the pelagic zone in the Gulf of Gdańsk*, Oceanologia, 55 (4), 859–886, http://dx.doi.org/10.5697/oc.55-4.859.

Witek Z., Humborg C., Savchuk O., Grelowski A., Łysiak-Pastuszak E., 2003, *Nitrogen and phosphorus budgets of the Gulf of Gdańsk (Baltic Sea)*, Estuar. Coast. Shelf Sci., 57 (1–2), 239–248, http://dx.doi.org/10.1016/S0272-7714(02)00348-7.

Witek Z., Ochocki S., Maciejowska M., Pastuszak M., Nakonieczny J., Podgórska B., Kownacka J. M., Mackiewicz T., Wrzesińska-Kwiecień M., 1997, *Phytoplankton primary production and its utilization by the pelagic community in the coastal zone of the Gulf of Gdańsk*, Mar. Ecol.-Prog. Ser., 148, 169–186, http://dx.doi.org/10.3354/meps148169.

Wright E. S., Yilmaz L. S., Noguera D. R., 2012, *DECIPHER, a search-based approach to chimera identification for 16S rRNA sequences*, Appl. Environ. Microb., 78 (3), 717–725, http://dx.doi.org/10.1128/AEM.06516-11.

Zhang Y., Jiao N., Cottrell M. T., Kirchman D. L., 2006, *Contribution of major bacterial groups to bacterial biomass production along a salinity gradient in the South China Sea*, Aquat. Microb. Ecol., 43 (3), 233–241, http://dx.doi.org/10.3354/ame043233.

Zhang Z., Schwartz S., Wagner L., Miller W., 2000, *A greedy algorithm for aligning DNA sequences*, J. Comput. Biol., 7 (1–2), 203–214, http://dx.doi.org/10.1089/10665270050081478.

Zwart G., Crump B. C., Kamst-van Agterveld M. P., Hagen F., Han S-K., 2002, *Typical freshwater bacteria: an analysis of available 16S rRNA gene sequences from plankton of lakes and rivers*, Aquat. Microb. Ecol., 28 (2), 141–155, http://dx.doi.org/10.3354/ame028141.

Supplementary information

Table S.1. Probes and competitors used in this study

Probe name	Target group	Reference
EUB 338-I	*Eubacteria*	Amann et al. (1990)
EUB 338-II	Supplement to EUB338	Daims et al. (1999)
EUB 338-III	Supplement to EUB338	Daims et al. (1999)
NON 338	Control	Wallner et al. (1993)
Alf 968	*Alphaproteobacteria*	Neef (1997)
Bet 42a	*Betaproteobacteria*	Manz et al. (1992)
Gam 42a-Comp	Competitor for Bet42a	Manz et al. (1992)
Gam 42a	*Gammaproteobacteria*	Manz et al. (1992)
Beta 42a-Comp	Competitor for Gam42a	Manz et al. (1992)
HGC 69a	*Actinobacteria*	Roller et al. (1994)
ROS 537	*Roseobacter clade*	Eilers et al. (2001)
CF 319a	*Bacteroidetes*	Manz et al. (1996)
RB-T 065	*Limnohabitans*	Šimek et al. (2001)
SAR11-441R	SAR11-clade	Morris et al. (2002)

Table S.2. Results of MANTEL test using sampling sites ZN2, E53, E54, E62

EnvProp	ρ (rho)	P
overall	0.692	0.001
Temp	0.481	0.016
Sal	0.603	0.001
PO_4	0.492	0.007
NO_2	0.531	0.006
NO_3	0.265	0.055
Si	0.507	0.004
NH_4	0.259	0.076
P_{org}	0.331	0.036
P_{tot}	0.221	0.086
N_{org}	0.614	0.002
N_{tot}	0.385	0.010
Chl *a*	0.223	0.079
PP	0.207	0.048
BPP	0.309	0.067
DOC	0.237	0.045
SUVA	0.320	0.036
Diatoms	0.880	0.001
Chlorophyta	0.207	0.097
Cryptophyceae	0.787	0.001
Cyanophyta	0.251	0.074
Coscinodiscus sp.	0.781	0.001

Table S.3. 16S rRNA clone, their phylogenetic affiliation and the corresponding in silico terminal restriction fragment (iTRF) (station E54). There were 86 good quality bacterial clones. TRF > 100 nt. One access number (Ac. No.) per TRF. Ac. no of clones with TRF < 100 nt not shown. B, brackish; M, marine; F, freshwater; N, nonaquatic

16S rRNA gene sequences	Phylogenetic affiliation	No. of clones	TRF [nt]
	Actinobacteria		
	Acidimicrobiaceae, CL500-29 marine group	11 (M)	
KF596583		1	243
KF596551		3	244
	Sporichthya hgcl	6 (F)	
KF596585		3	191
KF596574		1	233
KF596520		2	235
KF596581	*Sporichthya* ACK-M1	1 (F)	233
	Microbacteriaceae, *Candidatus* Aquiluna	2 (F, M)	
	Bacteroidetes		
KF596527	*Cytophagia*, *Cyclobacteriaceae*, uncult.	2 (F)	197
KF596562	*Cytophagia*, *Rhodothermaceae*, uncult.	1 (M)	152
KF596595	*Cytophagia*, *Reichenbachiella*,	1 (M)	139
	Flavobacteriaceae, uncultured	3 (M)	
KF596589		1	811
KF596609		1	812
KF596588	*Flavobacteriia*, *Croceitalea*	1 (M)	204
	Flavobacteriia, marine group NS3a	1 (M)	
KF596556	*Flavobacteriia*, *Owenweeksia*	1 (M)	236
KF596542	*Flavobacteriia*, marine group NS9	1 (M)	456
	Flavobacteriia, *Fluviicola*	1 (F)	
KF596544	*Sphingobacteriia*, *Chitinophagaceae*, uncult.	1 (F)	198
	Cyanobacteria		
	Synechococcus, subsection I	4 (F)	
KF596515		1	188
KF596607		1	190
KF596563		1	191
KF596516		1	193
KF596593	*Synechococcus*_2, subsection I	1 (B)	190
	Planctomycetes		
KF596513	*Planctomycetaceae*, uncultured	4 (B, F)	137

Table S.3. (*continued*)

16S rRNA gene sequences	Phylogenetic affiliation	No. of clones	TRF [nt]
	Proteobacteria		
	Alphaproteobacteria		
	SAR11Chesapeake-Delaware Bay	25 (B)	
KF596555		1	143
KF596545		1	144
KF596517		9	208
KF596536		4	209
KF596521		1	210
KF596514		1	211
KF596576		1	251
	Alphaproteobacteria SAR11 surface 1	2 (M)	
KF596608		1	173
	Rhodospirillaceae AEGEAN-169	2 (M)	
KF596553	*Roseobacter* DC5-80-3	1 (B; M)	250
KF596537	*Rickettsiales* uncultured	1 (N)	208
	uncult. *Alphaproteobacteria*	1 (M)	
	Betaproteobacteria, OM43	1 (F)	
	Alcaligenaceae MW-UniP1	2 (F)	
	Comamonadaceae BAL58	2 (B)	
KF596578		1	152
KF596531		1	153
KF596538	*Gammaproteobacteria*, OM182	3 (M)	257
KF596573	*Gammaproteobacteria*, OM60 NOR5	2 (M)	152
	Oceanospirillaceae Pseudospirillum	1 (M)	
	Verrucomicrobia		
KF596560	OPB35 soil group	1 (F)	162