Abundance and Taxonomic Diversity of Bacteria Inhabiting the Sediment-Water Interface in a Marine Harbor Channel

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Abstract – The abundance of bacteria inhabiting the sediment-water interface and their taxonomic composition were determined with the fluorescence in situ hybridization (FISH) method in a marine harbor channel in Ustka. Among bacteria inhabiting the studied layer *Gammaproteobacteria* ($1.4 \text{ cells } 10^8 \cdot \text{dm}^{-3}$) and *Cytophaga-Flavobacterium* ($1.1 \text{ cells } 10^8 \cdot \text{dm}^{-3}$) dominated. *Vibrio* and *Pseudomonas* represented only a small fraction of the total cell counts. All taxonomic groups of studied bacteria show significant positive correlation between their abundance. The total bacterial number varied from 3.3 to $23.5 \text{ cells } 10^8 \cdot \text{dm}^{-3}$ and their biomass oscillated from 39.4 to $282.4 \mu\text{g C} \cdot \text{dm}^{-3}$. This parameter differed along horizontal profiles, while there were no significant differences in the abundance of the studied taxonomic groups among the study sites of the channel in Ustka. The total number of bacteria as well as the abundance of bacterial phylogenetic groups were subject to seasonal fluctuation in the studied water basin.

Keywords – abundance, bacteria, marine harbor channel, sediment-water interface, taxonomic diversity

1. Introduction

The sediment-water interface (SWI) is an environmental and ecological boundary layer between overlying water and sediment in all water basins (Christian and Lind 2007; Chai et al. 2016). In natural waters, the sediment-water interface is the site where gradients of physical, chemical and biological properties are the greatest (Santschi et al. 1990; Accornero et al. 2002). In natural waters, the sediment-water interface is the site where gradients in physical, chemical and biological properties are the greatest. In natural waters, the sediment-water interface is the site where gradients in physical, chemical

and biological properties are the greatest. In natural waters, the sediment-water interface is the site where gradients in physical, chemical and biological properties are the greatest. SWI is a bio-reactive biotope, which plays a key role in biogeochemical reactions, such as the flux of detrital materials carried through the water column and their incorporation within the sediment record (Wu et al. 2001). These reactions include such processes as dissolution, degradation, mineralization and demineralization, deposition, sorption and desorption, migration, transformation, oxidation and reduction, and enzymatic hydrolysis (Christian and Lind 2007; Zhu et al. 2016). All these processes may strongly influence both water quality and the biota inhabiting SWI (Viaroli et al. 2004). Therefore the sediment-water interface is an important site where cycling of elements between sediments and overlying water is regulated and controlled (Zhu et al. 2016). In SWI large quantities of organic matter delivered from the water column and sediment are accumulated (Queiroz et al. 2004). The main stream of organic matter accumulated in the sediment-water interface originates from photosynthetically produced phytoplankton and microphytobenthos as well as dead phyto- and zooplankton and different anthropogenic pollutions (Bhaskar and Bhosle 2008; Divya et al. 2010). High concentration of organic matter in SWI stimulates optimal conditions for the development of many organisms such as microorganisms, phytoplankton, phytobenthos, macroalgae, vascular plants, meiofauna and macrofauna taxa (Daumas 1990; Snelgrove et al. 2000). Among organisms inhabiting the sediment-water interface the most numerous and those with high productivity and enzymatic activity are microorganisms, mainly aerobic and anaerobic bacteria with different metabolic potential (Xia et al. 2014; Wei et al. 2016). These prokaryotic

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organisms play a key role in ecological functions and processes such as regulation, assimilation, accumulation, export, decomposition, degradation, enzymatic hydrolysis, re-mineralization and transformation of the largest part of organic matter accumulated in SWI (Bucci et al. 2014; Wei et al. 2016). According to Tanase et al. (2009) and Liu et al. (2012), in order to fully understand the role of bacteria in the functioning of the sediment-water interface, it is necessary not only to determine their secondary production, enzymatic activity, respiration processes, morphological differentiation and physiological properties but also to determine their abundance and taxonomic diversity. The role of bacterial population in the functioning of SWI was studied by early researchers including Christian and Lind (2007), Ivanov et al. (2013), Dann et al. (2014), and Chai et al. (2016). However, to our knowledge, no studies are available on the taxonomic structure of bacteria inhabiting the sediment-water interface, in particular a specific water reservoir such as a marine harbour channel. Therefore, the main objective of this study was to determine phylogenetic diversity of bacteria and their abundance in SWI in the marine harbour in Ustka along a horizontal water profile and during different seasons using the fluorescence *in situ* hybridization (FISH) technique. This technique allows for the gradual revealing, identification and quantifying of different bacterial groups and also the description of the spatio-temporal dynamics of bacterial community structure in a variety of freshwater and marine ecosystems (Alonso et al. 2010; Lefort and Gasol 2013).

2. Material and Methods

Study area and site description

This study was carried out in a marine harbour channel that is the estuarine part of the Ślupia River (Poland). The studied channel is 40.5 m wide and about 6 m deep, and is located in the vicinity of the port in Ustka ($54^{\circ}35.2\text{N}$, $16^{\circ}21.2\text{E}$) (Fig. 1). The port in Ustka covers an area of 29.3 ha and its main functions are fishery, transport and marine tourism (Christowa et al. 2007). The studied harbour is limited by two breakwaters of about 300 m in length from the Baltic Sea. Selected chemical and bacteriological parameters of the sediment-water interface in the channel are presented in Table 1, and a detailed method of description regarding their determination is given by Perliński (2015).

The sediment-water interface samples planned for further bacteriological analyses were collected from the marine harbour channel at four sites (Fig. 2).

Site 1 - located on the border between the Ślupia River and the studied channel (freshwater zone),

Site 2 - situated in the central part of the channel (brackish water zone),

Site 3 - located in one of the water basins called the coal basin (brackish water zone),

Site 4 - situated at the site where the channel enters the sea, i.e., near the heads of breakwaters (marine zone).

Fig. 1. Map of localization the study marine harbour channel in Ustka

Table 1. Values of selected chemical and microbiological parameters in sediment-water interface in the harbour channel in Ustka (Perliński 2015)

Parameters	st. 1		st. 2		st. 3		st. 4	
	Mean	Range	Mean	Range	Mean	Range	Mean	Range
N-NO ₃ (mg·dm ⁻³)	46.0	27.5–68.8	43.2	18.3–86.3	45.1	24.6–96.6	44.2	19.2–101.9
N-NH ₄ (mg·dm ⁻³)	8.7	3.5–14.9	12.3	8.6–15.5	14.6	8.9–25.4	20.9	10.1–47.4
Cl ⁻ (mg·dm ⁻³)	396.8	48.5–751.9	5774.1	4828.7–6822.2	2590.3	398.9–5944.2	6009.0	5393.5–7386.6
O ₂ (mg·dm ⁻³)	7.7	6.6–10.9	7.7	3.6–1.5	7.6	5.2–9.8	7.8	5.4–9.8
pH	7.3	7.0–7.5	7.3	6.9–7.6	7.3	7.0–7.5	7.5	7.4–7.6
Organic Matter (mg·dm ⁻³)	0.2	0.1–0.3	2.4	1.7–3.1	0.8	0.3–1.1	3.0	2.1–3.9
Protein (μg·dm ⁻³)	21.7	15.4–29.8	20.3	16.5–24.2	21.9	16.9–26.9	24.9	18.5–31.9
Lipids (μg·dm ⁻³)	111.7	26.2–238.9	183.0	106.5–330.5	76.3	32.9–129.3	386.6	197.8–624.9
Carbohydrates (μg·dm ⁻³)	73.1	29.8–113.3	79.4	21.7–128.8	60.3	43.1–94.3	56.6	21.8–97.6
Number of heterotrophic bacteria (CFU 10 ⁶ ·dm ⁻³)	23.4	3.2–91.7	23.4	2.3–79.2	24.5	4.0–79.2	19.2	1.5–62.7
Bacterial production (μg C·dm ⁻³ ·h ⁻¹)	90.4	11.3–220.7	50.9	4.4–170.7	63.7	8.7–181.9	24.4	7.1–80.3

Fig. 2. Location of the sampling sites

Sediment-water interface sampling

The precise location of each sampling site was taken with a GPS receiver installed on board a tugboat. The water samples were collected during autumn (A) in 2012 and winter (W), spring (SP) and summer (S) in 2013. After localization of the site with an echosounder, the sediment-water interface samples were collected with a horizontal van Dorn water sampler adapted for the collection of the samples in flowing

water (Christian and Lind 2007; Ivanov et al. 2013). Prior to sampling, the sampler was rinsed with distilled sterile water and ethyl alcohol. The collected water samples were transferred to sterile glass containers using a drain valve. Then the samples were transported to the laboratory in ice containers at a temperature that did not exceed 7°C. The time between the collection of the samples and bacteriological analyses usually did not exceed 3 hours.

Determination of phylogenetic groups of bacteria inhabiting the sediment-water interface and their abundance

It has been well-documented (Cottrell and Kirchman 2000; Franklin et al. 2005; Hervas and Casamayor 2008; Stolle et al. 2011) that the most frequently studied taxonomic groups of bacteria inhabiting the marine coastal zone are such taxa as *Cytophaga-Flavobacterium*, *Gammaproteobacteria*, *Marine alpha-cluster*, *Pseudomonas* and *Vibrio*. Therefore, in the present study we also focused on phylogenetic analyses of these taxonomic groups. Bacterial phylogenetic groups and their cell abundance in the sediment-water interface of the studied channel were determined by the fluorescence in situ hybridization (FISH) technique using fluorescence-labelled, rRNA targeted oligonucleotide probes. The probe sequences, hybridization conditions and references used in this study are given in Table 2. For the determination of selected phylogenetic groups of bacteria, 20 cm³ of each water sample was fixed with formaldehyde (final concentration 4%) and filtered through white polycarbonate filters with a diameter of 47 mm and a pore size of 0.2 μm. The filters were removed from the filtration apparatus, rinsed with sterile distilled water, air

Table 2. The probes used in FISH

Oligonucleotide probe	Polygenetic target	Probe sequence	Reference
EUB338	Bacteria	5'-GCTGCCTCCCGTAGGAGT-3'	Amman et al. (1990)
CF319a	<i>Cytophaga-Flavobacterium</i>	5'-TGGTCCGTGTCTCAGTAC-3'	Manz et al. (1996)
GAM42a	<i>Gammaproteobacteria</i>	5'-GCCTTCCCACATCGTTT-3'	Manz et al. (1992)
ROS537	<i>Marine alpha-cluster</i>	5'-CAACGCTAACCCCTCC-3'	Eilers et al. (2001)
PAE997	<i>Pseudomonas</i>	5'-TCTGGAAAGTTCTCAGCA-3'	Alm et al. (1996)
GV-841	<i>Vibrio</i>	5'-AGGCCACAACCTCCAAGTAG-3'	Eilers et al. (2000)

dried and stored at -20°C until the analysis in the laboratory. FISH hybridization was performed according to Glöckner et al. (1996) and Walczak and Brzezinska (2010). Prior to hybridization, the thawed filters were cut into small sections. The filter sections were placed on glass slides and covered with 20 µl of a hybridization solution containing 0.9 M NaCl, 20 mM Tris-HCl (pH 7.4), 0.01% sodium dodecyl sulfate (SDS), variable (0–35%) formamide concentrations and 50 ng of CY-3-labeled oligonucleotide. Hybridizations were carried out at 46°C for 2 h in an equilibrated sealed moisture chamber. After hybridization, the filters were transferred to a wash solution containing 20 mM Tris HCl (pH 7.4), 5 mM EDTA, 0.01% 10% SDS and a recommended concentration of NaCl determined by formamide concentration from a hybridization buffer. After hybridization, cells were stained with sterile 50 µg of DAPI (4,6-diamidino-2-phenyl-indol) (Fluka) per cm³ for 15 min in the dark, then rinsed with 70% ethanol and air-dried on Whatman 3M paper. The filters were placed on a glass slide, and mounted with a 4:1 mix of Citifluor to minimize bleaching. Hybridized and DAPI stained cells were counted using an OLYMPUS BX-41 epifluorescence microscope equipped with a camera (Color View III) and fitted with a CY-3 filter (Omega optic Inc) and DAPI filter (UV 2A) at a magnification of 1000x. In all cases, bacteria were counted in 20 different fields with a minimum of 200 cells. The abundance per 1 dm³ of water was calculated according to the formula proposed by Zimmermann and Meyer-Reil

(1974). Enumeration results were always corrected by subtracting signals observed with the nonsense probe NON338. According to Fuhrman and Azam (1982), bacterial counts are converted to bacteria biomass using the converting factor of $1.2 \cdot 10^{-10} \cdot \text{dm}^{-3}$.

Statistical analysis

The standard deviation (SD), coefficient of variation (CV), and coefficient of dispersion (CD) were determined according to Velji and Albright (1986). The correlation between the levels of the studied bacteriological parameters was assessed with the Spearman rank order test. The significance of differences in bacteriological parameters among the seasons and sites was assessed by a two-way ANOVA and Kruskal-Wallis tests according to Incera et al. (2003). In order to group the analyzed parameters according to their specific properties, the cluster analysis (the Ward method) and Euclidean distance were applied according to StatSoft Inc.

3. Results

The total number of bacteria (TBN) in the sediment-water interface of the marine harbour channel varied from 3.3 to 23.5 cells $10^8 \cdot \text{dm}^{-3}$ (average 7.4 cells $10^8 \cdot \text{dm}^{-3}$), while their biomass ranged from 39.4 to 282.4 µg C·dm⁻³ (average 89.3 µg C·dm⁻³) during the annual cycle (Table 3). Phylogenetic analysis of the bacterial community showed that among five studied taxonomic bacterial groups the dominant ones throughout

Table 3. Total number and biomass bacteria and abundance phylogenetic groups bacteria in studied marine channel *·µg·dm⁻³

Specificity	Statistical parameters				
	Mean ($10^8 \text{ cells} \cdot \text{dm}^{-3}$)	Range ($10^8 \text{ cells} \cdot \text{dm}^{-3}$)	SD	CV [%]	CD
Total bacteria number (TBN)	7.4	3.3–23.5	5.0	67.0	354.1
Bacterial biomass*	89.3	39.4–282.4	61.6	67.0	42.5
<i>Cytophaga-Flavobacterium</i> (CF)	1.1	0.4–2.4	0.5	47.8	25.2
<i>Gammaproteobacteria</i> (GMP)	1.4	0.7–2.8	0.5	39.8	21.4
<i>Marine alpha-cluster</i> (MAC)	0.9	0.3–1.8	0.4	47.8	25.2
<i>Pseudomonas</i> (P)	0.4	0.2–0.7	0.1	31.7	4.4
<i>Vibrio</i> (V)	0.4	0.05–0.7	0.2	60.2	13.5

Fig. 3. Horizontal variations of total number of bacteria in sediment-water interface (average from the pooled data of all seasons). Vertical bars represent standard deviation, $n = 16$

Fig. 4. Abundance studied taxonomic groups bacteria inhabiting sediment-water interface in horizontal profile harbour channel in Ustka (average from the pooled data of all seasons). Vertical bars indicate standard deviation, $n = 16$

the year cycle were the *Gammaproteobacteria* subclass ($1.4 \text{ cells } 10^8 \cdot \text{dm}^{-3}$) and *Cytophaga-Flavobacterium* group ($1.1 \text{ cells } 10^8 \cdot \text{dm}^{-3}$). *Vibrio* and *Pseudomonas* represented only a small fraction ($0.4 \text{ cells } 10^8 \cdot \text{dm}^{-3}$) of bacteria identified in the studied estuary (Table 3).

Data presented in Figure 3 show the differences in the total bacterial number along the spatial profile of the studied harbour channel. The maximum values ($10.8 \text{ cells } 10^8 \cdot \text{dm}^{-3}$) of TBN in the sediment-water interface were noted at the site located on the border between the Słupia River and the channel

Fig. 5. Seasonal dynamics change of total bacteria number and taxonomic groups (average from the pooled data of four sites). Vertical bars indicate standard deviation, $n = 16$. Legends: TBN-Total number of bacteria; GMP-*Gammaproteobacteria*; CF-*Cytophaga-Flavobacterium*; MAC-*Marine alpha-cluster*; P-*Pseudomonas*; V-*Vibrio*

(freshwater zone-site 1). The lowest ($4.3 \text{ cells } 10^8 \cdot \text{dm}^{-3}$) total numbers of bacteria in SWI were noted in the marine zone (site 4). The abundance of phylogenetic groups of bacteria in SWI along the horizontal profile of the studied channel is presented in Fig. 4. It is evident that besides *Cytophaga-Flavobacterium*, regarding which maximum values were recorded at site 1, a very small fluctuation in the abundance of other taxonomic groups among the study sites of the marine harbor in Ustka was noted.

The results of our study showed that the total numbers of bacteria as well as the abundance of bacterial taxonomic groups were subject to seasonal fluctuation (Fig. 5). The highest total number of bacteria was recorded during summer. In other seasons, the number of these organisms was two-fold lower than in summer and remained at a similar level. *Gammaproteobacteria*, *Cytophaga-Flavobacterium* and *Marine alpha-cluster* were the most abundant in summer, while their number was lowest in winter. The number of the genera *Pseudomonas* peaked in spring and the highest number of the genera *Vibrio* was observed in winter and spring. The minimum number of both these groups was recorded in autumn.

The relationships between the polygenetic groups of bacteria in the sediment-water interface are given as a correlation matrix (Table 4). In SWI, we noted a significant positive correlation between the abundance of *Flavobacterium-Cytophaga* and *Marine alpha-cluster* ($r = 0.72$) and *Gammaproteobacteria* ($r = 0.49$). A significant positive correlation in the abundance of other groups of bacteria in this water layer was found between *Marine alpha-cluster* and *Gammaproteobacteria* ($r = 0.68$) and also *Pseudomonas*

($r = 0.48$) and between *Pseudomonas* and *Vibrio* ($r = 0.47$).

By grouping the results by the seasons and sites the factorial Fisher and Kruskal -Wallis tests were carried out to detect significant differences between the total number of bacteria and abundance of five studied phylogenetic groups (Table 5). The abundance of *Gammaproteobacteria*, *Marine alpha-cluster* and *Vibrio* differed significantly between the studied seasons. In our study, there were no statistically significant differences in the abundance of the studied taxonomic groups among the study sites of the marine harbour channel in Ustka.

The results of a hierarchical dendrogram analysis of the studied taxonomic groups showed two distinct clusters (Fig. 6). The first cluster (1) comprised two taxonomic groups: *Pseudomonas* and *Vibrio*, while the second cluster (2) comprised *Gammaproteobacteria*, *Marine alpha-cluster* and *Flavobacterium-Cytophaga*.

Fig. 6. Cluster analysis of particular enrichment factors performed for all studied phylogenetic groups bacteria (parameters applied: Euclidean distance, full binding)

Table 4. Correlation coefficient studied bacteriological parameters in sediment-water interface ($n=16$), Significance level (p) is indicated by asterisks * p -value < 0.05 , ** p -value < 0.01

	<i>Cytophaga – flavobacterium</i>	<i>Gamma-proteobacteria</i>	<i>Marine alfa cluster</i>	<i>Pseudomonas</i>	<i>Vibrio</i>
<i>Cytophaga – flavobacterium</i>					
<i>Gammaproteobacteria</i>	0.49*				
<i>Marine alfa cluster</i>	0.72**	0.68**			
<i>Pseudomonas</i>	0.19	0.08	0.48*		
<i>Vibrio</i>	0.07	-0.05	-0.01	0.47*	

Table 5. Analyses of 2-way-ANOVA and the Kruskal – Willis non-parametric test of variance in the studied taxonomic groups, due to site and season. Significance level (p) is indicated by asterisks ** $p < 0.01$, *** $p < 0.001$, ns-non-significant F-Fisher test, H-Kruskal-Wallis test

Source of variation	TBN		<i>Cytophaga – flavobacterium</i>		<i>Gamma-proteobacteria</i>		<i>Marine alfa cluster</i>		<i>Pseudomonas</i>		<i>Vibrio</i>	
	H	p	H	p	F	p	F	p	F	p	F	p
Season	4.79	ns	6.79	ns	9.06	**	12.16	***	2.42	ns	16.59	***
Site	7.39	ns	2.02	ns	0.15	ns	0.10	ns	1.09	ns	0.12	ns

4. Discussion

In all aquatic ecosystems the basic parameters used to describe the structure of bacteriocenosis populations, besides the level of metabolic and respirometric activity, are the total bacterial number and their taxonomic diversity (Ivanov et al. 2013; Xia et al. 2014). The knowledge of bacterial taxonomic composition is important for the understanding of global and local patterns and the role that bacteria play in the overall processes in all water basins (Bowman and McCaig 2003; Liu et al. 2012).

The total number of bacteria inhabiting the sediment-water interface in the marine harbour channel varied from 3.3 to $23.5 \cdot 10^8$ cells·dm⁻³. This range was similar with those reported by Tholosan and Bianchi (1998) in the sediment-water layer in NW Mediterranean (5.5 – $29.2 \cdot 10^8$ cells·dm⁻³), in the eastern Kara Sea from the region of the Yenisei estuary (1.7 – $2.5 \cdot 10^7$ cells·dm⁻³) (Ivanov et al. 2013), in Port Noarlunga in South Australia ($3.7 \cdot 10^5$ – $1.45 \cdot 10^7$ cells·dm⁻³) (Dann et al. 2014) and the harbour channel of the Buffalo River (2.7 – $16.0 \cdot 10^8$ cells·dm⁻³) (Pettibone et al. 1996).

Phylogenetic diversity of bacterial community inhabiting the sediment-water interface in the marine harbor in Ustka showed that *Gammaproteobacteria* dominated among the studied taxonomic groups. This result corresponds to the results of earlier studies on the surface microlayer and subsurface water carried out by Perliński (2015) in this water basin. This taxonomic group is among three classes of the phylum *Proteobacteria* comprising gram-negative aerobic and anaerobic, and also chemoheterotrophic bacteria (Franklin et al. 2005; Hunter et al. 2006). *Gammaproteobacteria* represent a large phylogenetic group of cosmopolitan species such as *Cohwellia*, *Alteromonas*, *Acinetobacter*, *Pseudoalteromonas*, *Shewanella*, *Marinobacter*, *Helomonas* and *Psychrobacter* (Bowman and McCaig 2003; Zhou et al. 2009). The dominance of this taxonomic group of bacteria in water basins has been reported by Cottrell and Kirchman (2000), Simanato et al. (2010) in water and by Xia et al. (2014), Galand et al. (2016) in sediment. A large number of *Gammaproteobacteria* in the sediment-water interface in the channel in Ustka most probably is due to the fact that those bacteria as a group are involved in many biodegradation processes, suggesting their importance in organic matter decomposition in SWI (Tanase et al. 2009; Zeng et al. 2011). Many bacteria from this taxonomic subclass have particular gene generating high proteolytic activity (Zhou et al. 2009; Alonso et al.

2010). In the sediment-water interface of the studied marine harbour channel, protein concentration (15.4 – 31.9 µg·dm⁻³) and percentage of proteolytic bacteria (37–55%) were high (Perliński 2015) and, consequently, that might induce the high abundance of *Gammaproteobacteria* observed in our study.

Apart from *Gammaproteobacteria*, the *Cytophaga-Flavobacterium* group was also an abundant phylogenetic group inhabiting the sediment-water interface of the studied water basin. Members of this taxonomic group are found in large numbers in all marine and freshwater basins (Feng et al. 2009; Liu et al. 2012). The genome analysis of *Flavobacteria* isolates has identified high numbers of genes encoding the synthesis of extracellular hydrolytic enzymes (Xu et al. 2003; Taylor et al. 2014). This suggests that this bacterial assemblage plays an important role in biochemical aerobic degradation processes of complex polymeric organic matter in water ecosystems (Tanase et al. 2009; Walczak and Brzezinska 2010; Liu et al. 2012). For this reason, the *Cytophaga-Flavobacterium* group has been characterized as metabolically highly active and able to degrade many high molecular weight compounds, mainly proteins, carbohydrates and lipids (Hempel et al. 2008), which were recorded in high concentrations in the sediment-water interface of the studied channel (Table 1).

Bacteria of *Marine alpha-cluster* group were comparatively less abundant in the sediment-water interface. Several studies (Sorokin 1995; Bowman et al. 1997; González et al. 1997) have shown that this taxonomic group comprises marine bacteria. Taking this fact into account, it seems reasonable that the maximum abundance of this bacterial group in the studied harbour channel was recorded in its most saline part (site 4). Similar results were reported by González and Moran (1997) in the estuary of the Altamaha River on the eastern coast of the United States.

Similar to the surface microlayer and subsurface water (Perliński 2015) of the studied harbour, the genera *Vibrio* and *Pseudomonas* represented only a small fraction of organisms in the sediment-water interface. Our results correspond to the findings on bacterial taxonomic composition in the Takayama River (Kenzaka et al. 1998), in the Changjiang Estuary and the coastal area of the East China Sea (Feng et al. 2009) and the surface sediment of the Yellow River China (Xia et al. 2014). The small number of the genera *Vibrio* and *Pseudomonas* in freshwater and brackish sediment-water interface in the studied marine harbour channel is probably due to the fact that these bacteria are typical marine organisms, which

require salt for growth and metabolic activity (Langenheder et al. 2003; Riemann et al. 2008).

The results of our study indicated different TBN in the sediment-water interface along the horizontal profile of the marine harbor. The total number of bacteria inhabiting the studied layer was the highest in the freshwater zone (site 1) while the lowest was in the marine zone (site 4). This variation in the abundance of bacteria along the spatial profile of the channel in Ustka may be the result of changes in water salinity. According to Jiang et al. (2006) and Kouridaki et al. (2010), salinity, as a physiological stress factor, decreases cell-specific activity and growth efficiency and thus controls bacterial abundance. Alonso et al. (2010) and Lefort and Gasol (2013) observed a gradient of decreasing bacterial numbers with increasing salinity in water basins and this fact corresponds well with our results. Also, the estuarine character of the studied water basin that influences its hydrodynamics with steep physicochemical gradients due to the variability of freshwater and seawater input may impact on the total bacterial number along the horizontal profile (Webster et al. 2015; Wei et al. 2016).

Heidelberg et al. (2002) and Jorquera et al. (2004) reported the horizontal profile variation in the quantification of different bacterial taxonomic groups among marine and estuarine environments. According to Lefort and Gasol (2013) and Galand et al. (2016), spatial variation in the bacterial composition and diversity is determined mainly by origin, concentration, availability and degradability of organic matter, as well as the level of metabolic activity of microbial populations. In our study, we did not find any considerable variation in the abundance and diversity of taxonomic groups among the study sites in SWI of the marine harbour channel in Ustka. These results may suggest lack of differences in organic matter concentrations and their availability and also similar levels of metabolic activity of bacteria along the horizontal profile of the studied harbour.

Our research documented seasonal fluctuation in the total number of bacteria in the studied water basin. The highest total number of bacteria was noted in the summer season. According to Mary et al. (2006) and Takenaka et al. (2007), the main stimulus for cell-specific activity, growth efficiency and multiplication of bacteria in summer is the intensive development of phytoplankton and phytobenthos. The assimilates released by these organisms are immediately used by bacteria as food or an energy substrate which generates an increase in their number. According to Cottrell and

Kirchman (2000) and Takenaka et al. (2007), relatively high temperatures in summer are also an important environmental factor directly governing bacterial activity and resulting in their high abundance. Seasonal variation in the number of determined bacterial polygenetic groups in the sediment-water interface of the studied harbour channel was also shown by the results of the present study. This seasonal fluctuation in the number of bacterial taxonomic groups was earlier documented in the Delaware drainage basin (Hullar et al. 2006), Liu et al. (2012), in the Changjiang Estuary and East China Sea and in the Arabian Sea (Bandekar et al. 2016). Mary et al. (2006) and Bucci et al. (2014) suggested that a variety of different environmental parameters (temperature, nutrient concentration, liability of organic matter, primary production and grazing bacteria) favoured different groups of polygenetic bacteria at different times of the year. *Gammaproteobacteria*, *Flavobacterium-Cytophaga* and *Marine alpha-cluster* were dominant in the water of the studied water basin in summer. These results correspond to the results of earlier studies in the surface microlayer and subsurface water carried out by Perliński (2015) in this water basin and data collected in the Elbe and Spittelwasser rivers (Brümmer et al. 2000) and in the northern Baltic Sea Pinhassi and Hagström (2000).

5. Conclusions

In conclusion, we hope that our study will enhance knowledge about the spatial and seasonal polygenetic diversity of bacteria inhabiting the sediment-water interface in marine harbour channels.

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