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ABUNDANCE OF LIVE AND DEAD CELLS OF BACTERIONEUSTON AND BACTERIOPLANKTON FROM THE SŁUPIA RIVER ESTUARY

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Abstract

The study was carried out in the estuarine part of the Słupia River, which, for the most part, comprises the harbour channel. The results of the present study showed that the total abundance of bacterioneuston was higher compared to bacterioplankton. In these two groups of bacteria, dead bacterial cells were dominant. The total number of bacteria, as well as the number of live and dead bacteria was similar in the entire horizontal profile. The abundance of live and dead bacteria showed distinct seasonal variation.

Key words: estuary, harbour channel, abundance, bacterioneuston, bacterioplankton

INTRODUCTION

Bacteria in water ecosystems play a key role in the microbial loop and all biogeochemical cycling processes (Quéric et al. 2004, Säwström et al. 2008). These organisms are active in the processes of organic matter decomposition and also are the principal route of nutrient cycling in all water basins (Hoppe et al. 2002, Davidson et al. 2004, Freese et al. 2006). Thus, the accurate determination of bacterial abundance in aquatic ecosystems, i.e., the total bacterial number, and distinguishing metabolically active from inactive (or nonliving) cells, are very important steps to understand the role of bacteria acting as recyclers of dissolved organic matter as only living and metabolically active cells mediate organic matter turnover (Falcioni et al. 2008, Senjarini et al. 2013). Various methods have been used to enumerate live and dead bacteria from environmental samples (Yokomaku et al. 2000). One the most commonly used method to distinguish live (active) from dead (inactive) cells is the LIVE/DEAD Baclight method (Leuko et al. 2004, Freese et al. 2006, Perliński et al. 2015, Kubera

and Donderski 2017). It allows not only rapid, simple and relatively precise counting of the total number of bacteria but also characterizing the physiological state of bacterial populations (Davidson et al. 2004, Bogdanova et al. 2014) and therefore it was applied in the present study. According to the LIVE/DEAD Baclight method, cellular and membrane integrity is considered to be one criterion distinguishing between live and dead bacteria. Live cells are assumed to have intact cell membranes that cannot be penetrated by some staining compounds, whereas dead cells are considered to have disrupted and/or broken membranes (Stiefel et al. 2015). The estimation of the number of live and dead bacteria in water basins is particularly important in surface microlayer because large number of bacterial cells accumulate in this ecotone. Therefore, the aim of this paper was to determine the abundance of live and dead bacterial cells inhabiting surface and subsurface water in the Słupia River estuary and their spatial and temporal variability.

MATERIAL AND METHODS

Study area and sampling

The present study was carried out in the estuarine part of the Słupia River, which, for the most part, comprises the harbour channel. By this channel, which is 40.5 m wide, about 6 m deep, and is limited by two breakwaters of about 300 m length from the open sea, the port in Ustka (54°35.2N, 16°21.2E) (Fig. 1) is located. This port covers the area of 0.3 km^2 and its main functions are fishery, transport and marine tourism (Christowa et al. 2007).

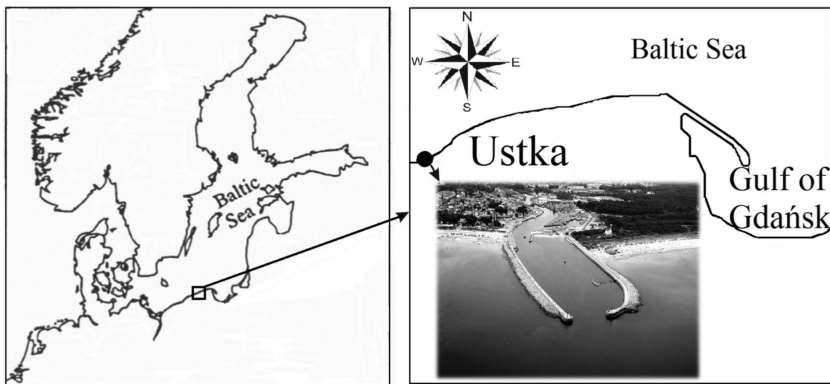

Fig. 1. Map of localization the study of the Słupia River estuary

The water samples for further bacteriological analyses were collected at four sites (Fig. 2).
Site 1 – located on the border between the Słupia River and the harbour channel,
Site 2 – located in the central part of the channel,
Site 3 – located in one of the water basins called the coal basin,
Site 4 – located at the site where the channel enters the sea, i.e., near the heads of breakwaters.

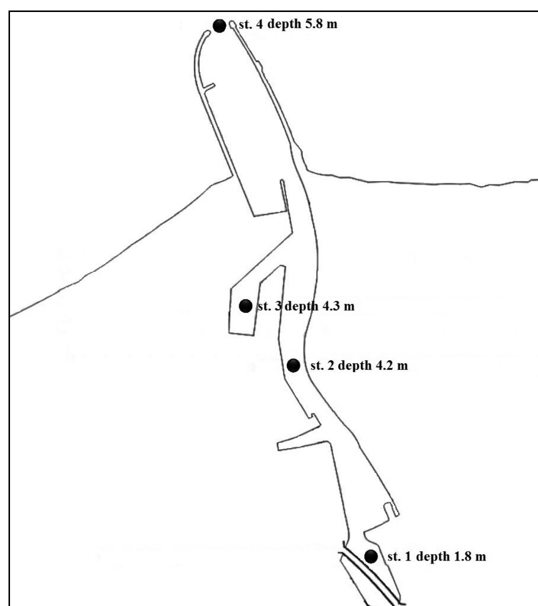

Fig. 2. Location of the sampling sites

The precise location of each sampling site was taken with a GPS receiver installed on the board of the tugboat. The water samples were collected during winter (w), spring (sp), summer (s) and autumn (a) from 2010 to 2013. The surface microlayer (SML) (thickness of 250-300 µm) was sampled with a 75 x 75 cm Garrett net (Garrett 1965) of 0.14 mm mesh size. The water collected in the net was scrapped off with the wiper and the sample was collected in a sterile bottle. The subsurface water (SSW) was sampled at about 0.5 m depth with a horizontal Van Dorn water sampler adapted for the collection of the samples in flowing water (Mudroch and MacKnight 1994). The water samples collected with the Van Dorn sampler were transferred to sterile bottles using drain valve. Prior to sampling, the Garret net and Van Dorn sampler were rinsed with distilled sterile water and ethyl alcohol. The collected samples of water were transported to the laboratory in the ice containers at the temperature that did not exceed 7°C. The time between the collection of the samples and bacteriological analyses usually did not exceed 3 hours. Some data on chemical parameters of the sampled water are given accordign to Perliński et al. (2017) in Table 1.

Determination of bacterioneuston and bacterioplankton abundance

To estimate the total number of neustonic and planktonic bacteria, and to distinguish between live and dead bacteria, we used the LIVE/DEAD staining kit supplied by the Molecular Probes company. Following the protocol of the producer, bacterial staining was applied to fresh, unfixed water samples. To prepare working solutions, two reagents supplied by the producer (component A and B) were dissolved in 5 cm^3 of the sterile, fluorescent water obtained from Fluka. After dissolving of two components, they were mixed with the studied water sample in a 1:1 ratio, and then were put into sterile tubes.

Table 1
Values of selected chemical parameters in Słupia River estuary (according to Perliński et al. 2017)

		st. 1		st. 2	
Parameters	Units	Mean	Range	Mean	Range
N-NO3	mg·dm^{-3}	7.26	1.37-15.98	8.49	3.65-15.66
N-NH4	mg·dm^{-3}	48.45	26.06-103.23	50.39	25.27-136.43
Cl-	mg·dm^{-3}	294.28	36.46-741.40	419.57	94.58-1250.28
O2	mg·dm^{-3}	7.05	4.76-10.42	7.44	4.17-11.11
pH		7.28	6.80-7.70	7.27	6.53-7.70
Organic matter	mg·dm^{-3}	0.45	0.30-0.70	0.79	0.30-1.70
Protein	μg·dm^{-3}	23.81	15.12-34.32	22.10	16.31-28.01
Lipids	μg·dm^{-3}	109.21	43.88-222.57	85.21	36.27-175.54
Carbohydrates	μg·dm^{-3}	72.31	31.27-135.11	75.46	21.87-154.96
		st.3		st. 4	
Parameters	Units	Mean	Range	Mean	Range
N-NO3	mg·dm^{-3}	13.56	3.9-48.43	11.68	4.16-22.3
N-NH4	mg·dm^{-3}	43.82	22.4-100.25	45.93	21.97-106.63
Cl-	mg·dm^{-3}	574.87	128.88-1306.12	1852.13	381.92-5909.11
O2	mg·dm^{-3}	6.81	5.63-7.61	7.06	5.21-9.13
pH		7.45	7.09-7.70	7.45	7.18-7.67
Organic matter	mg·dm^{-3}	0.68	0.40-0.90	0.89	0.20-2.30
Protein	μg·dm^{-3}	21.01	15.31-27.42	22.99	17.31-29.87
Lipids	μg·dm^{-3}	105.04	24.38-248.46	360.57	20.09-888.59

The obtained mixtures were vortex-mixed and incubated in the dark for 10-15 minutes. After incubation, the samples were again vortex-mixed, and filtered with Millipore apparatus through 0.2 μm pore size, 13 mm diameter black, polycarbonate filters. After filtration of the sample, the filter was washed with 1 cm^3 of distilled filtered water. The stained filters were dried, and then mounted on microscopic slides. The observations were performed using an epifluorescence microscope (Olympus BX-41) at magnification of x10 eyepiece and x100 objective lens, equipped with a filter block B-2A for blue light (EX450-490 excitation filter, DM510 dichroic mirror and BA520 barrier filter; excitation λ = 480/490 nm, emission λ = 500/635 nm). Bacteria were counted in 20 randomly selected fields of view from each layer, and each sampling site. Bacteria stained green were classified as “live”, whereas bacteria stained red were classified as „dead”. Following Quéric et al. 2004, the number of live and dead bacteria, and their sum given as their total number (TBN) were calculated according to the formula proposed by Zimmermann and Meyer-Reil (1974).

RESULTS

Bacterial abundance, i.e., their total number, and the number of live and dead cells were presented in Figure 3. These data showed that the total number of bacterioneuston was higher than bacterioplankton. In the surface and subsurface water layers, dead bacterial cells were dominant. Moreover, a higher percentage difference

between live and dead bacterial cells was noted in the SML compared to the SSW. In bacterioneuston, the percentage of live cells (33%) was twofold lower than that of dead bacterial cells (67%). In bacterioplankton, living cells constituted 42%, while dead cells – 58% of TBN.

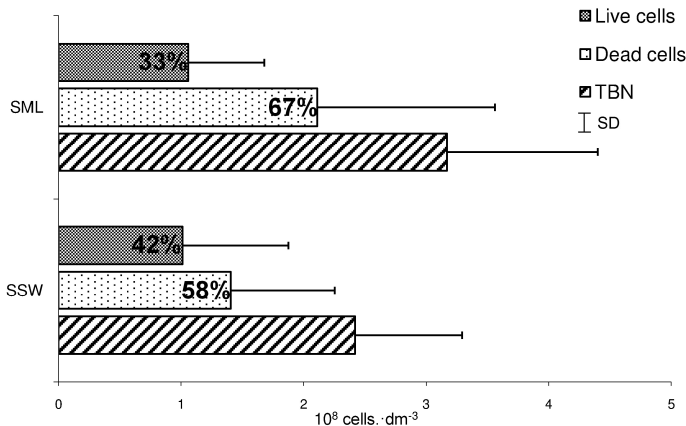

Fig. 3. Total bacteria number and the number of live and dead cells in surface (SML) and subsurface water (SSW) layers (average from the pooled data of all sites and season). Vertical bars represent standard deviation of the mean, n = 8

According to the data shown in Figure 4, the total bacterial number along the studied horizontal profile was similar. The amount of bacteria with disintegrated cytoplasmic membrane at particular study sites was higher than the number of metabolic active bacterial cells. Similar to TBN, the number of live and dead bacteria along the studied horizontal profile did not show significant oscillation.

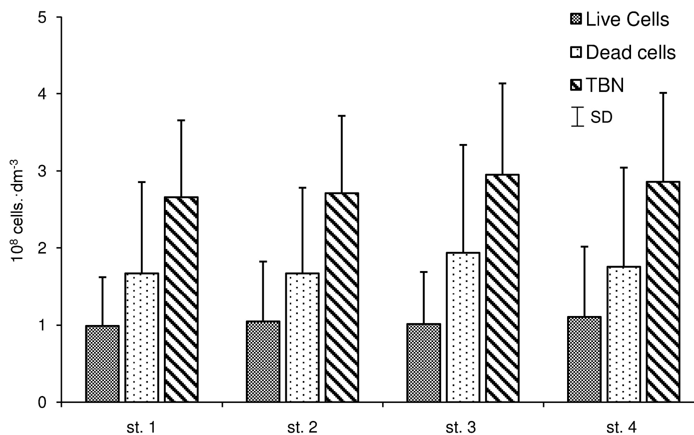

Fig. 4. Spatial variations abundance live and dead cells bacteria in different parts of studied estuary (average from the pooled data of all water layers and seasons). Vertical bars represent standard deviation of the mean, n = 8

The results of the present study (Fig. 5) indicated distinct seasonal variation in the abundance of live and dead bacteria. In the case of living and dead bacteria cells, seasonal fluctuations in their abundance were observed. The maximum number of live bacterial cells in the surface microlayer ($2.3 \cdot 10^8$ cells$\cdot$dm^{-3}) and subsurface water ($3.1 \cdot 10^8$ cells$\cdot$dm^{-3}) was noted in summer of 2011, while the minimum number in autumn of 2012 (SML – $0.4 \cdot 10^8$ cells$\cdot$dm^{-3}, SSW – $0.3 \cdot 10^8$ cells$\cdot$dm^{-3}). The data on seasonal abundance of neustonic and planktonic bacteria with disrupted cytoplasmic membrane indicated three maxima in their abundance, i.e., in spring of 2011 ($4.0 \cdot 10^8$ cells$\cdot$dm^{-3}) and 2013 ($4.7 \cdot 10^8$ cells$\cdot$dm^{-3}), and summer of 2012 ($3.6 \cdot 10^8$ cells$\cdot$dm^{-3}). The lowest number of dead bacterial cells in the surface microlayer and subsurface water was noted in summer (0.6-$0.7 \cdot 10^8$ cells$\cdot$dm^{-3}) and autumn of 2011 (0.5-$0.8 \cdot 10^8$ cells$\cdot$dm^{-3}).

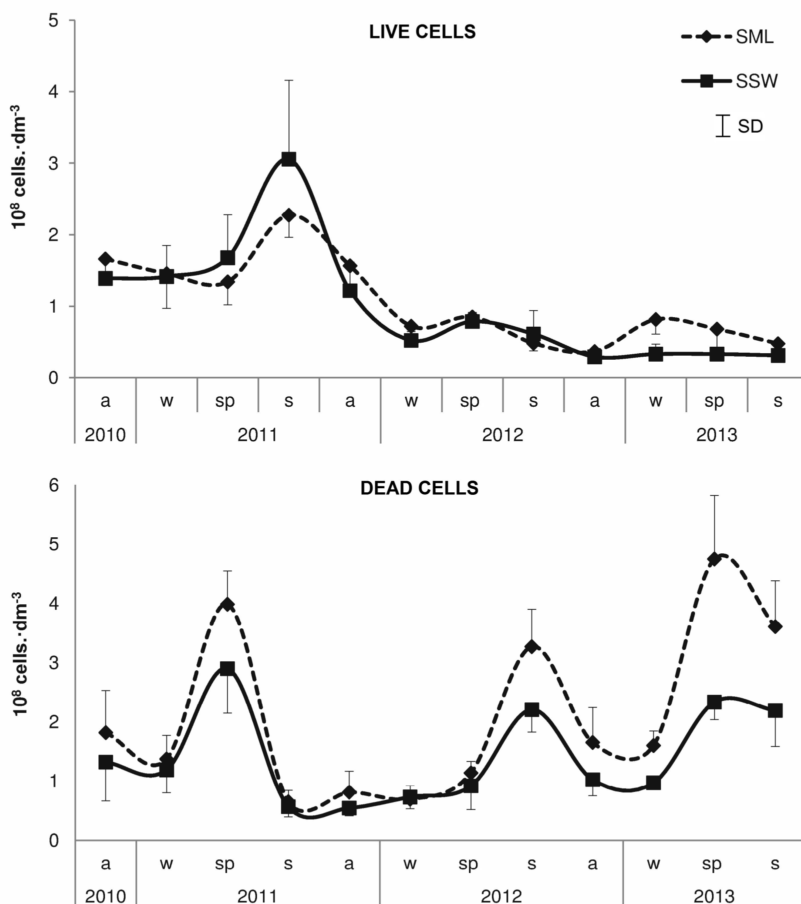

Fig. 5. Seasonal dynamics change of number of live and dead cells bacteria in water of the Słupia River estuary (average from the pooled data of all water layers and all sites). Vertical bars indicate standard deviation, n = 8

DISCUSSION

The results of the present study on the abundance of bacteria inhabiting analysed water layers in the harbour channel in Ustka showed that this parameter was higher in the border zone: air-water compared to the subsurface water layer. Similar results were reported in other studies on the abundance of bacterioneuston and bacterioplankton in freshwater bodies (Donderski et al. 1999, Zdanowicz 2009), estuaries (Mudryk et al. 2003, Santos et al. 2009), and marine ecosystems (Mudryk et al. 1991, Stolle et al. 2010). High accumulation of neustonic bacteria in water bodies results from several environmental factors. Probably high concentration of organic matter in the border zone: air-water stimulates the optimal conditions for development of microbial communities (Walczak and Donderski 2005, Cunliffe et al. 2013). According to Wurl and Obbard (2004), a factor generating high abundance of bacterioneuston are also excretions and secretions of phyto- and zooneuston. They are mostly composed of carbohydrates, proteins and lipids which are very actively used as nutrient and energetic substrates by bacteria inhabiting the surface microlayer (Hillbricht-Ilkowska et al. 1997). Another factor that contributes to accumulation of high amount of bacteria in the surface microlayer is the presence of specific, extracellular compounds of hydrophobic character (mucopolysaccharides, glycoproteins, phosphocholines and lecithin polymers) in these organisms, which enables them to adhere to the surface microlayer (Walczak and Donderski 2005). Moreover, several bacteria that are able to move actively may migrate to the surface microlayer from the deeper water layers due to chemotaxis (Maki 1993). Hervas and Casamayor (2008) also point at the fact that the surface microlayer may be enriched in bacteria due to solid and liquid precipitation. Another factor generating such numerous abundance of neustonic bacteria is the fact that the surface microlayer which is in direct contact with atmosphere, provides aerobic bacterioneuston with optimal oxygen concentration, which due to diffusion penetrates from atmosphere to the border zone air-water (Walczak 2002). The results of our study showed that dead cells were the dominant fraction of bacteria in the water of the harbour channel in Ustka, and they accounted for 58-67% of the total bacterial number in that bacteriocenosis. This pattern was observed in the surface and subsurface water layers, as well as in the entire horizontal profile. These results indicate that only a small fraction of the bacterial community of this water basin was metabolically active and contributed to respiration, remineralisation and the microbial loop (Davidson et al. 2004). These data correspond to the results of the studies conducted in rivers (Freese et al. 2006), lakes (Berman et al. 2001, Haglund et al. 2003), coastline regions (Choi and Sherr 1996), and marine ecosystems (Luna et al. 2002, Zampino et al. 2004). Low number of live bacterial cells according to the studies by Haglund et al. (2003), Davidson et al. (2004) and Freese et al. (2006) may result from their grazing by bacteriovorus protozoa, mainly flagellates. There is evidence indicating that flagellates and other micrograzers preferentially graze metabolically active cells (Gonzáez et al. 1993, del Giorgio et al. 1996), which would tend to decrease the proportion of active cells in bacterial population. Another factor decreasing the number of live bacterial cells in the studied harbour channel are fluctuations in the water salinity level, which

cause osmotic shock of bacterial cells. This process may frequently lead to lysis of bacterial cells, and their death afterwards (Painchaud et al. 1995).
The results of our study showed that the number of dead bacteria was higher in the surface microlayer than in the subsurface water layer. Also in vertical profile of the Atlantic Ocean, the percentage of live cell decreased from ca. 65 in the surface water to 27 in the subsurface water. The rate of the decrease in the amount of live cells with depth was similar to that of bacterial production and respiration (Falcioni et al. 2008). High number of dead neustonic bacteria may be also due to the inhibitory effect of sunlight radiation, especially the shortest wavelength fraction of UV radiation, on these organisms. Solar UV radiation (UVR, 290 to 400 nm) causes cellular damage on different cell targets, including nucleic acids, proteins and lipids, which may end up in mutations, cell inactivation and death (Alonso-Saez et al. 2006, Santos et al. 2012, Perliński et al. 2015). According to Haglund et al. (2003), also viruses found to be numerous in the surface microlayer can be important regulators of bacterial mortality in this water layer. It has been suggested than viruses can cause up to 30% mortality of bacteria in aquatic ecosystems (Bratbak and Heldal 2000, Säwström et al. 2008).
The results of the present study documented seasonal variation in the abundance of live and dead bacteria in the water of the studied channel. As a rule, high percentage of dead bacteria, particularly in bacterioneuston, was noted in spring and summer, when bacteriostatic or bactericidal UV radiation is the most intense. The decline in the number of live bacterial cells during intensified insolation was also observed by Davidson et al. (2004), Lamy et al. (2006) and Freese et al. (2006). Beside negative influence of solar radiation, Mudryk et al. (1999) indicated that also excretion of bacteriostatic and bactericidal substances particularly by cyanobacteria and chlorophyta during spring and summer algal bloom could be another factor causing increased bacterial mortality.

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LICZEBNOŚĆ ŻYWYCH I MARTWYCH KOMÓREK BAKTERIONEUSTONU I BAKTERIOPLANKTONU W ESTUARIUM RZEKI SŁUPI

Streszczenie

Badania bakteriologiczne przeprowadzono w estuariowym odcinku rzeki Słupi będącym kanałem portowym. Wodę z czterech stanowisk badawczych pobierano z błony powierzchniowej oraz warstwy podpowierzchniowej. W badanych próbach przy użyciu mikroskopu epifluoroscencyjnego oznaczono ogólną liczebność bakterioneustonu i bakterioplanktonu oraz liczebność komórek bakterii żywych i martwych. Uzyskane wyniki badan wykazały, że bakterie neustonowe były liczniejsze niż bakterie planktonowe. W obu warstwach dominowały martwe komórki bakterii. W profilu horyzontalnym stwierdzono nieomal homogenne liczbowe występowanie badanych organizmów. W bakterioustonie wysoki odsetek martwych komórek, odnotowano wiosną i latem, kiedy promieniowanie UV jest najbardziej intensywne.