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ABUNDANCE AND PRODUCTION OF BACTERIA IN A MARINE BEACH (SOUTHERN BALTIC SEA)

ABSTRACT: The paper presents the results of the study of abundance, biomass, mean cell volume and secondary production of bacteria inhabiting a marine-bay sandy beach, at southern Baltic Sea coast (Sopot) in summer season. The differences of bacteriological parameters among sites across beach horizontal profile were determined. Maximal value of the total bacteria numbers (8.59 ± 0.73 × 10^7cells g^{-1} dw), biomass (15.2 ± 4.4 μg C g^{-1} dw) and cell volume of bacterium (0.056 ± 0.011 μm^3) was noted at the waterline and bacterial secondary production was highest in the dune (172.3 ± 86.6 μg C g^{-1} dw d^{-1}) and at the waterline (119.9 ± 40.5 μg C g^{-1} dw d^{-1}). Marked differences in the level of bacteriological parameters between surface and subsurface sand layers were estimated. In the sea, at the waterline and in the middle of the beach higher numbers of bacteria, their biomass and secondary production were found in the surface (0–1 cm) than in the subsurface (5–10 cm) sand layers. A reverse situation was observed in the dune.

KEY WORDS: sandy beach, total bacteria number, secondary production, spatial and vertical profile, southern Baltic Sea

1. INTRODUCTION

Marine sandy beaches are regions of transition between the land and the sea, and as such they are subject to significant influences from both ecosystems (Vieira *et al.* 2001, Bayed 2003, Fabiano *et al.* 2004). In coastal ecosystems, sandy beaches play a very important role in energy flow and the transfer of nutrients (Jędrzejczak 1999, Urban-Malinga and Opaliński 1999, Covazii Harriague *et al.* 2006). The sand of a marine beach can be regarded as a gigantic cleaning filter because of the large amounts (10–91 $m^3 m^{-1} d^{-1}$) of water passing through it in the accumulation – splash zone (Nair and Bharathi 1980, McLachlan 1989, Heymans and McLachlan 1996). While the seawater is being filtered, large amounts of dissolved organic matter (DOM) and particulate organic matter (POM) are adsorbed on the surface of the sand grains. This organic matter consists mainly of phytobenthos assimilates, products washed and leached out of the seaweed, animal faeces produced mainly by meio- and macrofauna and seabirds, and remains of marine plants and animals (Koop and Griffiths 1982, Brown and McLachlan 1990, Jędrzejczak 1999).

Organic matter reaching marine beaches can also originate from rivers and the surrounding land (Węsławski *et al.* 2000). According to Urban-Malinaga and Opaliński (1999) as much as 1100 mg of organic C m^{-2} could be introduced daily into the interstitial system of marine beach. The average organic matter content of sediment in a sandy beach oscillated from 855 to 1174 g C m^{-2} (Covazzi Harriague *et al.* 2006). Such great concentration of organic matter in marine beaches is further utilized by interstitial organisms and returns to the sea in the form of nutrients; therefore, in most beaches the interstitial system functions as a biological filter that mineralises organic matter and thus cleanses the surf water (Urban-Malinga and Opaliński 2001, Kotwicki *et al.* 2002).

The key role in the processes of destruction and transformation of organic matter accumulated in sandy beach ecosystems is played by bacteria (Mudryk *et al.* 2001, Podgórska and Mudryk 2003, Fabiano *et al.* 2004, Misic and Fabiano 2005, Misic and Covazzi Harriague 2007). According to Koop and Griffiths (1982) bacteria inhabiting sandy beaches due to their usually high numbers, deep penetration in the sediment and rapid turnover rates are likely to make a large contribution to the matter turnover and energy flow in these ecotones. According to Koop *et al.* (1982) about 70% of organic matter reaching a marine beach ecosystem is mineralized by bacteria and converted into their own biomass. It has been estimated that bacteria which constitute over 90% microorganisms occur in psammonn can mineralize approximately 100 kg of organic carbon and over 4 kg of organic nitrogen per 1 m^2 per year (Brown and McLachlan 1990).

In water basins the rate of decomposition of DOM and POM depends mainly on the abundance of bacteria and their productivity (van Duyl and Kop 1994, Chróst and Siuda 2006). Thus, elucidating the major determinants of bacterial abundance and

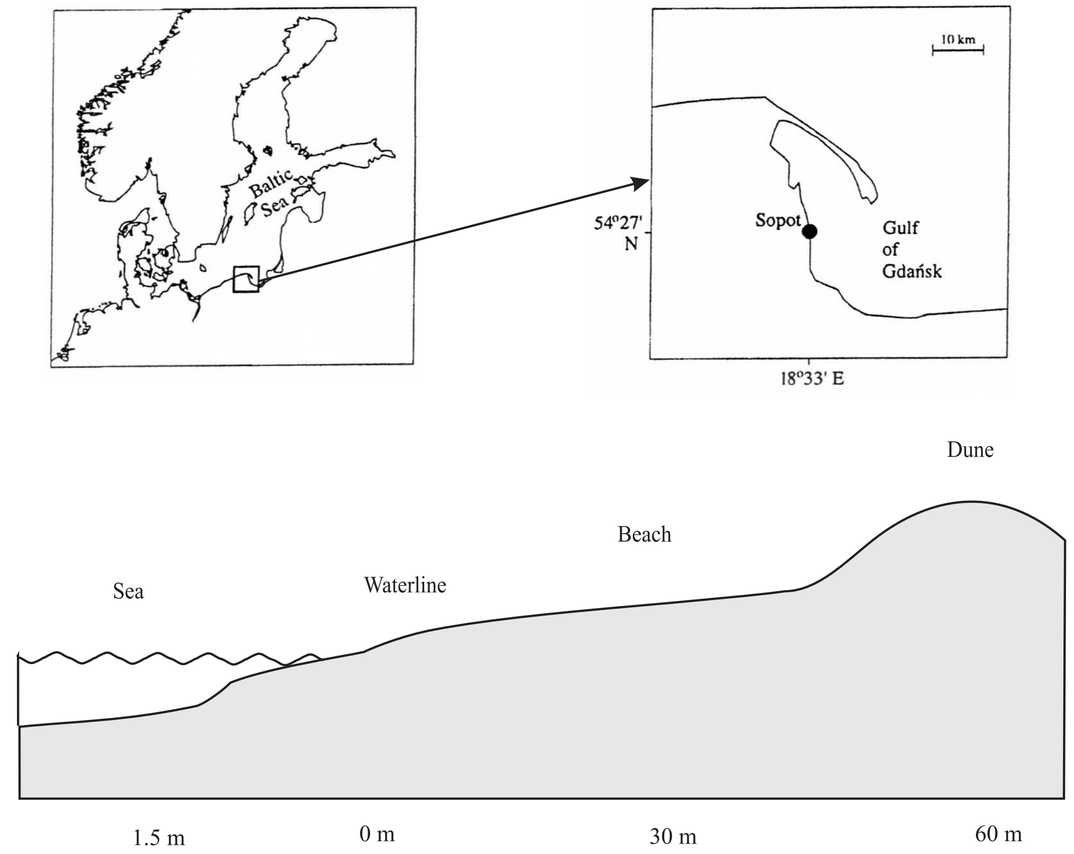

Fig. 1. Map of the study sites. Location of the sampling sites in the profile beach.

their production in various marine beaches is a significant ecological issue. Unfortunately, few studies on sandy beach bacteria have been carried out previously (Misic and Fabiano 2005, Mudryk and Podgórska 2007) and we know relatively little about the total number of bacteria and their rate of secondary productivity in marine beach ecosystems; the understanding of those problems is still incomplete and fragmentary. Therefore, the aim of this paper was to determine horizontal (zonation) and vertical profiles of the changes in the abundance and rate of secondary production of bacteria inhabiting marine sandy beach located in southern Baltic Sea in summer season.

2. STUDY AREA

The study was carried out on a non-tidal sandy beach near Sopot, Poland (54°27`N, 18°33`E) (Fig. 1) localized in the west of the Gulf of Gdańsk – one of the open bays of the southern Baltic, characterized by high levels of pollution and eutrophication (Haque *et al.* 1996, Urban-Malinga and Opaliński 2001). The studied beach represents a dissipative beach type with longshore bars and troughs; it has a slope of 7° and a width of about 50 m. The beach is exposed to moderate waves (average 0.3) from NE (Kotwicki *et al.* 2002). In general, fine and medium – grained sand (mean grain diameter 0.3 mm) predominates (Jędrzejczak 1999, Urban-Malinga and Opaliński 2001). The salinity of the overlying water ranges from 4 to 8 PSU. The water temperature is highest in August (22°C) and lowest in February (1°C) (Kotwicki *et al.* 2002). The Sopot beach, with its surf zone is ideal for holidaymarkes and a suitable area for recreational activities. It is frequented by tourists whose density in summer reaches 30 persons per 100 m^2; about 3000 people walked daily along the water line (Węsławski *et al.* 2000).

Sand samples were collected from the beach during July 2001. A transect was marked along a beach profile perpendicular to the shoreline and four sampling sites were located along this transect (Fig. 1). Site 1 was located under the water at a depth of about 1m, approximately 1–1.5 m from the waterline, site 2 was situated at the waterline, site 3 lay in the middle of the beach, at 30 m distance from the waterline, and site 4 lay in a sheltered place among the dune, at 60 m distance from the waterline.

Five sand core samples per site were taken with a hand-operated sampler (length – 30 cm, inner diameter – 15 cm). In the field, the sampled sand cores were divided horizontally into two sections: 0–1 cm and 5–10 cm, and placed in sterile glass jars which were put into containers with ice and transported to the laboratory. The time between sample collection and performance of bacteriological analyses did not exceed 2–3 h. Some physicochemical properties of sandy beach under study in summer season are shown in Table 1.

3. METHODS

The total numbers of bacteria (TBN) were established with the acridine orange direct method (AODC) according to Hobbie

Table 1. Values of selected physicochemical parameters in the sandy beaches of Sopot (data by Jankowska 2001 and Podgórska 2002).

Parameters	Units	Mean	Range
Air temperature	°C	18.3	15.0–23.0
Water temperature	°C	15.7	15.0–17.0
Sand temperature	°C	22.0	15.0–40.0
Chloride	mg g^{-1} dw	0.293	0.011–0.738
Organic matter	mg g^{-1} dw	4.1	2.2–7.2
Organic carbon	mg g^{-1} dw	3.5	1.9–6.5
Total nitrogen	μg g^{-1} dw	1.75	0.80–2.0
Total phosphorus	μg g^{-1} dw	0.240	0.037-0.486

et al. (1977). Sand samples (about 2 g) were collected in sterile plastic tubes, added to 5 cm^3 of 0.2 μm filtrated and sterile artificial seawater with prefiltrated formaldehyde (2% final concentration) and tetrasodium pyrophosphate solution (10 mM final concentration). In order to desorb the cells of bacteria from the sand grains the samples were sonificated (Sonifer Vibra Cell VC 130PB, 3 mm microtip sonic probe) three times for 30 seconds over ice bath and washed three times with sterile artificial seawater. Two cm^3 subsamples were filtrated using black-stained polycarbonate filters (Millipore, 0.2 μm pore size) and stained for 5 min with fresh acridine orange solution (final concentration of 0.5%). Bacteria were counted and sized under an epifluorescence microscope (Olympus 60BX) performed at 1000 × magnification. At least 200 cells or 20 microscope fields were counted in each of three replicate preparations. Bacterial abundance was reported as number of cells g^{-1} dry weight. Bacterial cell volume (V) was estimated by measuring 50 cells per sample using a micrometric ocular (Walton & Backett, Proton G 12). For the bacterial biomass (BB) estimations of carbon conversion factor of 0.35 pg C per μm^3 was used (Bjørnsen 1986). Bacterial abundance and biomass were normalized to dry weight after desiccation (60°C, 24 h).

The secondary production of bacteria (BP) in the sediment was determined by measuring the rate of incorporation of methyl-[^{3}H] thymidine ([^{3}H] TdR) into the bacterial DNA (Furhman and Azam 1982, Simon and Azam 1989). Sediment samples (about 2 g) for measuring thymidine incoropration were transferred to test tubes and mixed with 2 cm^3 sterile artificial seawater. To each test tube, 25 μl aqueous solution 20 μCi [^{3}H] TdR (NEN Life Science Products 6.7 Ci mmol specific activity) was added. Samples were incubated in the dark for 2 hours *in situ* temperature. After this period, the incubation was stopped by adding 57 μl 1 N NaOH to the samples. Controls were treated with NaOH prior to isotope addition. Samples were heated at 100^0C for 2 hours, centrifuged at 5000 x g for 10 min and supernatants were acidified with 3.5 cm^3 cold 1 N HCl. The acidified solutions were cooled for 45 min on ice and the precipitate immediately collected on a Millipore filter. After washing three times with 5 cm^3 cold 5% trichloracetic acid (TCA) the DNA was hydrolysed in 2 cm^3 5% TCA. Filters were then dissolved in 1 cm^3 of ethyl acetate before counting and placed in scintillation vials (Packard) with 10 cm^3 LCS – cocktail (Packard, Filter-Count). After 24h, the samples were radio-assayed in a Packard TRI-CRAB 2100TR liquid scintillation counter. Incorporation of isotope was converted to bacterial production (BP) according to Moriarty (1988), using conversion factor of 1 × 10^{18} cells produced per incorporation of 1 mole of thymidine.

The obtained data were statistically analysed. Statistical characteristics (standard deviation (SD), coefficient of variation (CV) were based on Velji and Albright (1986).

4. RESULTS

The total numbers of bacteria (TBN) in the sand beach varied from 3.43 to 9.11 × 10^7 cells g^{-1}dw (g^{-1} dw) (avg. 5.98 ± 2.07 × 10^7 cells g^{-1}w), and their biomass (BB) oscillated from 5.64 to 14.63 μg C g^{-1}w (avg. 10.78 ± 4.28 μg C g^{-1} dw) (Table 2). Data on the total numbers of bacteria and their biomass in the study beach indicate marked differences in the horizontal profile. The maximal values of TBN (8.59 ± 0.73 × 10^7cells g^{-1} dw) and BB (15.2 ± 4.4 μg C g^{-1} dw) were noted in the waterline. Minimal numbers of bacteria (3.96 ± 0.74 × 10^7 cells g^{-1}dw) and biomass (8.05 ± 3.06 μg C g^{-1} dw) were determined in the middle of the beach (Fig. 2).

Data presented in Table 2 show that the cell volume of bacterium (V) ranged from 0.040 to 0.065 μm^3 (avg. 0.051 ± 0.009 μm^3). Maximal average cell volume (0.056 ± 0.011 μm^3) characterized bacteria inhabiting waterline and minimal cell volume (0.048 ± 0.012 μm^3) bacteria cells which were isolated from the sea (Fig. 2).

Bacterial secondary production (BP) in the sand of the studied beach varied from 24.5 to 233.6 μg C g^{-1} dw d^{-1} (avg. 108.1 ± 61.4 μg C g^{-1} dw d^{-1}) (Table 2). In the studied zones of beach, differences in the level of bacterial production were estimated. The highest rates of secondary bacterial production were noted in the dune (172.3 ± 86.6 μg C· g^{-1} dw·d^{-1}) and waterline (119.9 ± 40.5 μg

C g^{-1}dw d^{-1}) whereas minimal ones (49.9 ± 35.9 μg C g^{-1} dw d^{-1}) were noted in the middle of the beach (Fig. 2).

The significance differences the values of bacteriological parameters between surface (0–1 cm) and subsurface (5–10 cm) sand layer were determined (Fig. 3). In the dune, a higher bacterial number, biomass and production were found in the subsurface (5–10 cm) than in the surface sand layer (0–1 cm). At other sites, a reverse situation was observed. Data presented in Figure 3 show that bacteria inhabiting surface layers were characterized by higher cell volume than bacteria inhabiting the subsurface layers. Only at the site localized in the sea, the volume of bacteria cell in the subsurface sand layer was 1.5 times higher than in the surface layer.

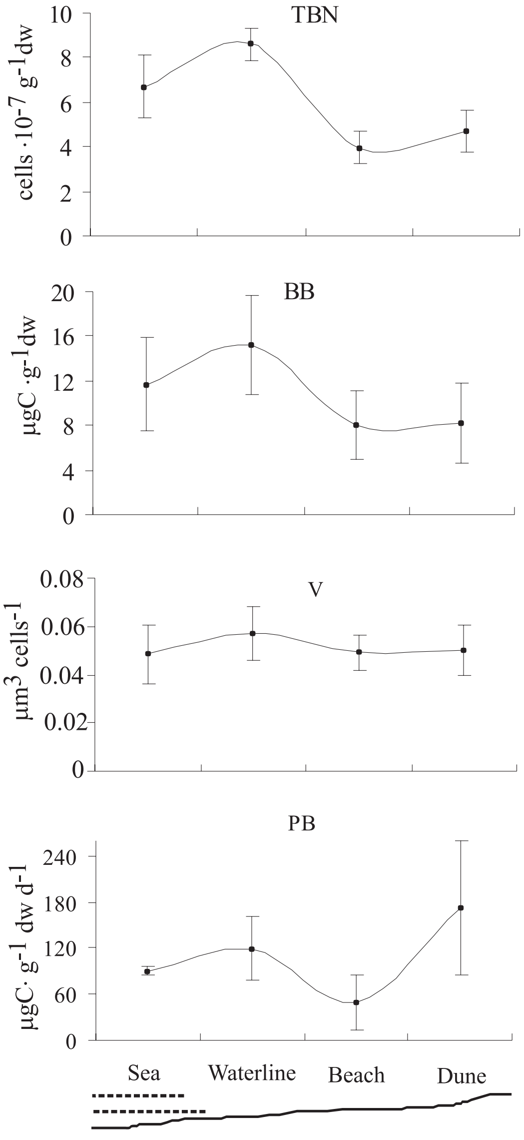

Fig. 2. Horizontal variations of bacteriological parameters in studied beach. Each point shows mean value in both studied sand layers. Vertical lines represented ±SD. Explanations: TBN – total bacteria numbers, BB – bacterial biomass, V – cell volume, BP – bacterial production.

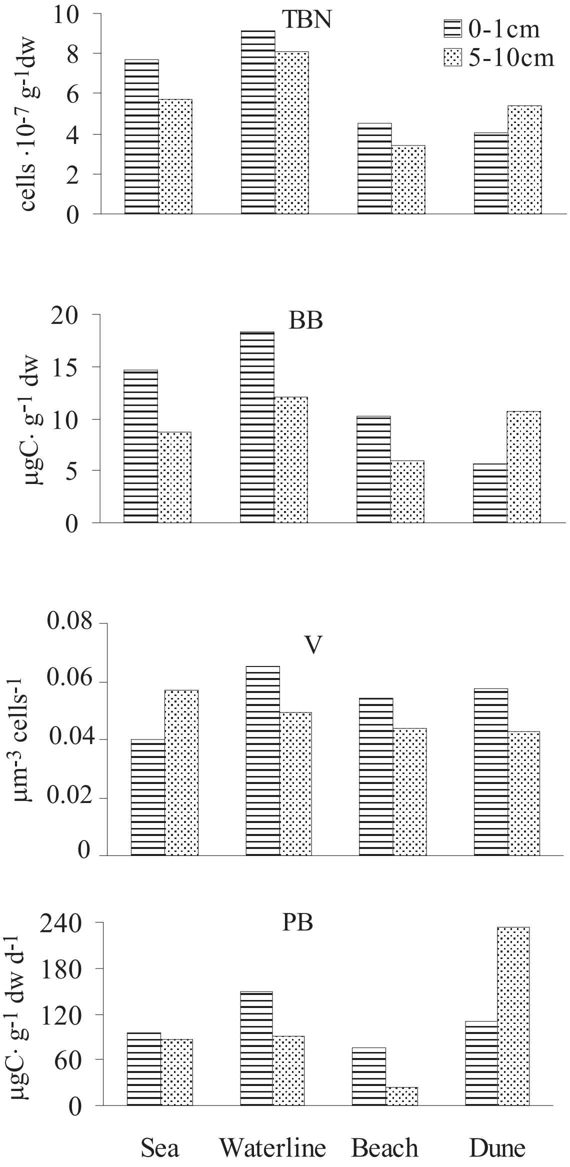

Fig. 3. Vertical profiles of the total bacteria numbers (TBN), bacterial biomass (BB), cell volume (V) and bacterial production (BP) in the surface (0–1 cm) and subsurface (5–10 cm) sand layers.

Table 2. Range and average values of selected bacterial parameters in sand (pooled data of all sites and layers)

Bacteriological parameters	Statistic parameters			
	Range	Average	±SD	CV (%)
Total numbers of bacteria (cells × 10^7 g^{-1} dw)	3.43–9.11	5.98	2.07	35
Bacterial biomass (μg C g^{-1} dw)	5.64–14.63	10.78	4.28	40
Bacterial cell volume ($μm^3$ $cell^{-1}$)	0.040–0.065	0.051	0.009	17
Bacterial secondary production (μg C g^{-1} dw d^{-1})	24.5–233.6	108.1	61.43	57
Bacterial secondary production (mg C m^{-2} dw d^{-1})	14.80–148.2	73.27	41.19	56

5. DISCUSSION

Bacteria inhabiting marine beaches play an important role as a main link between primary producers and higher trophic levels of the benthic food webs. Due to their small size, bacteria can respond quickly to the changes in abiotic and biotic environmental factors; their large numbers and productivity may significantly affect food web structures and survival strategies of higher trophic levels in marine beaches (Moreno *et al.* 2006).

Bacterial abundance in the sandy beach in Sopot varied from 3.4 to 9.1 × 10^7 cells g^{-1} dw. This range was consistent with ranges reported in other beaches, such as the sandy beaches of Nova Scotia (Canada) (1.9–7.3 × 10^7 cells g^{-1} dw) (Novitsky and MacSween 1989) and Collelungo beach (Italy) (0.2–15.0 × 10^7 cells g^{-1} dw) (Moreno *et al.* 2006) but lower (0.9–1.79 × 10^8 cells g^{-1} dw) than values obtained on beach a Baia Blu (Italy) (Misic and Covazzi Harrigue 2007). Biomass of bacteria inhabiting the sand of the studied beach oscillated from 5.6 to 14.6 μg C g $^{-1}$dw and the average cell volume ranged from 0.040 to 0.065 $μm^3$. Similar values (6.6–14.8 μg C g^{-1} dw), (0.055–0.110 $μm^3$) were reported by Misic and Fabiano (2005) in two sandy beaches of Arenzano (Italy).

The horizontal and vertical zonation of the distribution and metabolic activity of bacteria communities is a well known global phenomenon on marine sandy beaches (McLachlan and Jaramillo 1995, Olańczuk-Neyman and Jankowska 1998, Podgórska and Mudryk 2007). This zonation of the distribution and the rate of metabolic activity of bacteria is dependent upon different physico-chemical parameters (beach type, grain size, exposure to wave action, tidal level, the swash climate, composition of inorganic and organic matter, temperature, porosity, permeability, oxygen concentration, pH, salinity) as well as on biological factors such as availability of food or occurrence of bacteriovory organisms and viruses (Meyers 2000, Urban-Malinga and Opaliński 2002, Moreno *et al.* 2006). According to Nair and Bharathi (1980) and Koop and Griffiths (1982) the most important factor determining the numbers of bacteria in the sand of a marine beach is the supply and concentration of organic matter. The studies carried out by Pugh *et al.* (1974) and Novitsky and MacSween (1989) on British and Canadian beaches shown that the numbers of bacteria are much higher in this zone of the beach which is characterized by a high concentration of organic matter. Similarly, the present study has shown that on the studied Baltic beach in the waterline zone which was characterized by a constant inflow of nutrients from the sea caused by the waves, large amounts of organic matter are accumulated (8.5 mg g^{-1}dw) (Podgórska 2002) and the numbers of bacteria and their biomass were much higher than in other parts of the studied beach where the concentration of organic matter was lower (2.5–3.0 mg g^{-1}dw). Low numbers and biomass of bacteria recorded at the middle of the studied beach resulted possibly from the bacteria having been eaten by bacteriovory organisms, especially macrofauna and meiofauna, which, as has been shown by Haque *et al.* (1996) are very numerous in this part of the beach. According to Brown and McLachlan (1990) and Moreno *et al.* (2006) macrofauna, meiofauna and ciliates for which microorganisms, mainly bacteria, are a basic source of food and main energy input, are important factors controlling the numbers of bacteria on marine beaches.

Generally, investigations carried out in different beaches (Boucher and Chamroux 1976, Olańczuk-Neyman and Jankowska 1998, Mudryk and Podgórska 2007) demonstrated that in the vertical profile the greatest abundance of bacteria occurred in the top layer of sand, and decreased with depth. McLachlan *et al.* (1981) has suggested that bacteria actively assimilated easily removable components of organic matter in the top few centimeters of the sand, which stimulates rapid oxygen uptake and growth of bacteria. Data on the total numbers of bacteria in the studied beach also indicate that these organisms except in the dune occur more frequently in the surface than in the subsurface sand layers. This pattern of distribution results most probably from the fact that the concentrations of organic matter (Mudryk and Podgórska 2007) and oxygen (Urban-Malinga and Opaliński 2001) which generate optimal conditions for the development of bacteria – decrease with depth.

There is a general agreement that bacterial production is a major pathway of the flow of organic matter in many aquatic ecosystems (Chróst *et al.* 2000, Lemée *et al.* 2003, Misic and Covazzi Harriague 2007). Comparison of biotic components in sandy beaches Koop and Griffiths (1982) shows a total annual production ratio of macrofauna:meiofuana:bacteria of approximately 1:1:4.7. It is evident that bacteria assume paramount importance in the food web structure and energy flow in sandy beach ecosystem.

Bacterial production level in the sand of the studied beach oscillated from 15 to 148 mg C m^{-2} d^{-1} (Table 2). Similar, level (28–80 mg C m^{-2} d^{-1}) of secondary production on the Baltic beach near Kiel Bight (Germany) was noted by Meyer-Reil (1986) and Kemp (1987) (55 mg C m^{-2} d^{-1}) on sandy beaches of Oregon (north-eastern Pacific). A much higher (77–582 mg C m^{-2} d^{-1}) bacterial productivity than in this study was determined by Sündback *et al.* (1996) in a sandy bay of Saltö (Sweden) and Fallon *et al.* (1983) (100–800 mg C m^{-2} d^{-1}) in the sandy beaches on Sapelo in the Island Atlantic Ocean (USA).

Strong differences of bacterial production rates in horizontal and vertical profiles of the studied beach were demonstrated. Generally, except the dune, higher rates of bacterial production were determined in the surface than in subsurface sand layers. It seems that higher numbers of bacteria and organic matter concentration in the top sand layer (Podgórska 2002) is the main factor generating this high rate of bacterial production. In the deeper layers organic matter is less easily removed and is thus broken down much more slowly. In addition, light penetration measurements showed that the top of the sediment constitutes a euphotic zone in the sediment of the studied Baltic beach (Urban-Malinga and Wiktor 2003). Solar radiation in the surface sand layer can stimulate photochemical transformation of recalcitrant dissolved organic matter into such compounds as primary amines, amino acids and combined amino acids that are readily assimilated by bacteria, which in turn increased their productivity. In the deeper sand layer light penetration is blocked and photodegradation of organic matter is reduced. These observations suggested that in the top layer of sand, solar radiation may be instrumental in providing additional sources of nutrients available to bacterial productivity, as has been pointed out by Almeida *et al.* (2001). Additionally, the primary production of microphytobenthos and the burrowing activity of macro- and meiofaunal organisms as well as the breakdown of organic material into smaller particles probably act to enhance bacterial productivity in the surface sand layer (Urban-Malinga and Opaliński 2002).

Very high rate of bacterial production recorded in subsurface sand layer in the dune may have resulted from the accumulation of considerable amounts of organic matter, mainly in the form of humic substances, originating from decaying roots predominantly of grasses growing on the dune. As has been shown by Chróst *et al.* (2000), humic substances may be utilized by bacteria very intensively as a good source of food or energy. In coastal dunes McLachlan *et al.* (1996) estimated total primary production of 177–200 g m^{-2} $year^{-1}$ for the of scarce vegetation, and annual input of detritus as 162.8 g m^{-2} $year^{-1}$; it possibly stimulates an increased productivity of bacteria in the subsurface sand layers in marine dunes.

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