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Biological factor controlling methane production in surface sediment in the Polish part of the Vistula Lagoon

by

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Abstract

Due to the limited water exchange, lagoons are particularly prone to eutrophication. The consumption of oxygen in this process, coupled with simultaneous enrichment of bottom sediments with organic matter, reinforces the occurrence of anaerobic conditions and methanogenic growth. Methanogenic archaea activities cause depolymerization of organic compounds accumulated in sediments. As a result of such ecosystem transformation, methane might be produced and emitted from this basin. Chemical studies conducted in 2010 were focused on methane content in the surface bottom sediments in the Polish part of the Vistula Lagoon. The results showed that the highest methane concentration occurs in the southwestern part of this basin (6.45 mmol dm^{-3}), while the lowest one in the southeastern part (7.1 × 10^{-3} mmol dm^{-3}). Molecular studies were focused on specific methanogenic archaea gene identification. The comparison of nucleotide sequences of "mcrA gene" clones obtained from genomic DNA isolated from the Vistula Lagoon sediments indicates a similarity to the yet uncultivated archaea, but also to archaea from the *Methanosarcinales* and *Methanomicrobiales* orders.

Key words: methane, sediment, methane-forming bacteria, Vistula Lagoon, southern Baltic Sea

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Introduction

The Vistula Lagoon is the second largest lagoon in the Baltic Sea after the Curonian Lagoon. It has formed as a result of natural processes and is located partly in Poland and partly within the Russian Federation. The average depth of the basin is 2.7 m and the maximum depth, measured to the southeast of the Strait of Baltiysk, is 5.2 m. The basin is connected with the Baltic Sea by the Strait of Baltiysk (Sołowiew 1975; Witek et al. 2010).

The catchment area of the Vistula Lagoon borders on the Vistula River basin in the south and on the Niemen River catchment area in the east and north. The bottom sediments of this basin are closely related to its historical formation. The eastern part of the basin is mainly covered with silt formations, while the content of loam particles increases toward the north-west. Along the coast, sand sediments are found in strips of about 2 km in width, sparsely interspersed with silt. Typical sediments of the Vistula Lagoon are silt formations, characterized by plasticity and dark grey coloring. The thickness of silt close to the southeastern shores increases toward the sand bar, reaching up to 10 meters. Underneath the silt, peat layers are found at various depths (Nieczaj et al. 1975; Wypych et al. 1975; Witek et al. 2010; Kruk 2011).

The oxygen concentration in the Vistula Lagoon is determined by a number of factors, including mainly the course of biochemical processes, which are the most important for the decomposition of organic matter. The decomposition of algal mass is conducive to the depletion of oxygen and the synthesis of hydrogen sulfide in the near-bottom layers (Trzosińska & Żurawlewa 1975; Nawrocka et al. 2011). Grala et al. (2012) and Dębowski et al. (2016) indicate that algal biomass from the Vistula Lagoon effectively produces methane under anaerobic conditions. The presence of methane and methane-forming bacteria was already determined in the Polish part of the Baltic Sea (Brodecka & Bolałek 2011; Reindl & Bolałek 2012a,b; 2014), but there is limited data for lagoons that play an important role in the regional development and climate change.

High primary production, which provides a constant supply of organic matter to the sediments, as well as oxygen deficiencies create favorable conditions for the growth of methane-forming bacteria in the surface zone of bottom sediments. The performed research involved the determination of the potential for biochemical changes of organic compounds accumulated in sediments in the presence of a biological factor under anaerobic conditions. The aim of the presented studies was to determine the methane concentration in the Polish part of the Vistula Lagoon and the composition of microbial consortia from a single sampling campaign. The study also involved the taxonomic identification of the methane-forming bacteria.

Materials and methods

Collection of samples

Samples were collected from a 10 cm thick sediment layer of bottom sediments in the Polish part of the Vistula Lagoon using an Ekman grab sampler. The sampling was carried out from a MIR 2 watercraft during a voyage on 20-21 September 2010. The surface sediment layer was sampled at nine sampling sites, monitored as part of the National Environmental Monitoring Program (Fig. 1, Table 1).

Replicate bottom sediment samples (at least three samples) were collected for chemical analysis and processed in the way previously described by Reindl & Bolałek (2014) for seawater, with the following modification: we filled vials with 3 cm^3 of NaCl and 3 cm^3 of saturated sodium chloride solution and closed them under nitrogen atmosphere. Samples were collected using a cut-off syringe and placed in vials, which were immediately sealed with a rubber stopper and an aluminum cap.

Samples of benthic sediments for molecular analysis were collected in sterile vials made of synthetic material, ranging in volume from 100 cm^3 to 120 cm^3. The vials filled with sediment were put in a protective bag made of synthetic material, and then immediately placed in a refrigerator. The sediment samples were kept at a temperature not exceeding 5°C until molecular analysis, which began no later than 12 hours after sampling.

Chemicals

Pure hydrochloric acid, sodium chloride and chromatographically pure deionized water, supplied by MERCK, were used for chemical analysis. The following gases were used during chromatographic analysis: 99.995% pure helium and 99.9995% pure methane, both made by Linde.

The following commercial kits by A&A Biotechnology (Poland) were used for molecular analysis: "Genomic Mini AX Bacteria" for genomic DNA isolation, "2xPCR Master Mix Plus" for PCR reaction mixture, "Gel-Out" for mcrA gene isolation after agarose gel electrophoresis, and "Plasmid Mini AX" for plasmid DNA isolation, a DNA size marker and a DNA

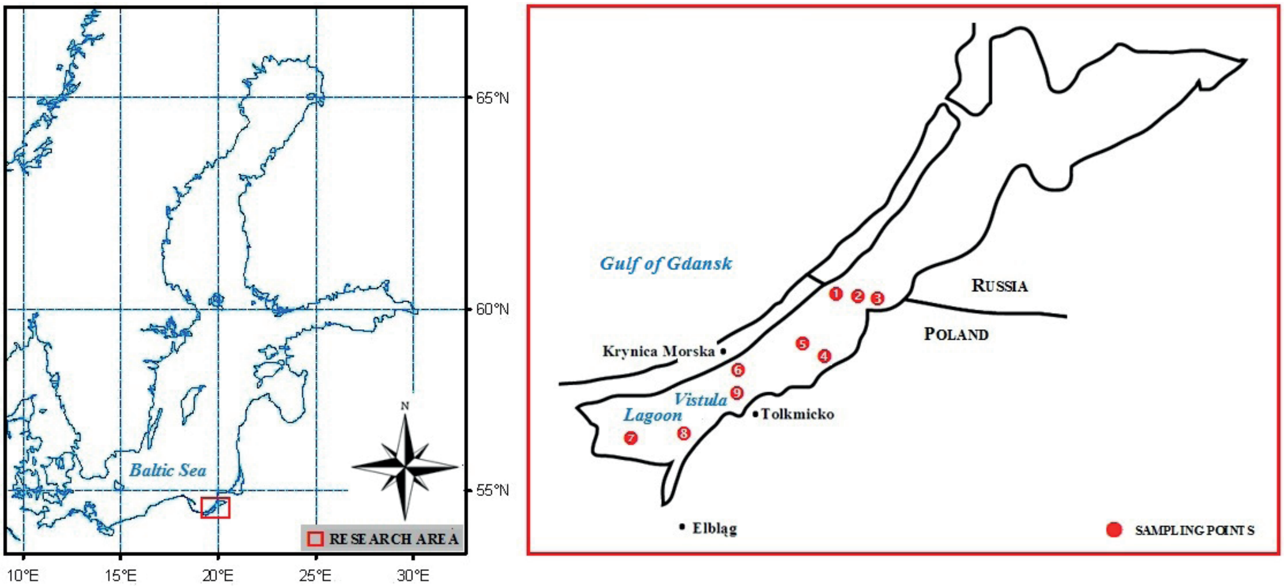

Figure 1

Location of the sampling sites in the Vistula Lagoon

thermostable polymerase WALK. The "CloneJET" PCR cloning kit from Fermentas, which contains a cloning vector named pJET1.2, was also used. Chemically competent *E. coli* cells (One Shot TOP10F') were supplied by Invitrogen. Primers of the mcrA gene for polymerase chain reaction (PCR), as previously described by Luton et al. (2002), were made to order by Sigma-Aldrich. The primers had the following genetic sequences:

5′-GGTGGTGTMGGATTCACACARTAYGCWACAGC-3′ [mcrAF]
5′-TTCATTGCRTAGTTWGGRTAGTT-3′ [mcrAR].

Chemical monitoring results

The data on temperature, transparency, oxygen and salinity in the Vistula Lagoon were obtained from the Polish Monitoring Program of the Vistula Lagoon water. All data, from sampling to the results obtained, were analyzed under ISO 17025 requirements.

Chemical analysis

The method of methane recovery from sediments consisted in changing the saturation in saline conditions as described elsewhere (Amouroux et al. 2002; Reindl & Bolałek 2014). The methodology used

Table 1

Location of sediment sampling sites in the Polish part of the Vistula Lagoon and near-bottom water parameters during sediment sampling

Location of sampling sites			water depth	temperature	transparency	oxygen	salinity
			m	°C	m	mg dm^{-3}	PSU
1	54°45′12.5″	19°66′87.5″	2.9	6.8	0.5	11.9	3.4
2	54°44′71.4″	19°72′46.4″	4.8	7.2	0.6	12.0	3.6
3	54°44′48.9″	19°76′05.8″	3.4	7.3	0.7	11.2	3.6
4	54°36′61.1″	19°66′64.2″	2.5	6.7	0.7	11.8	3.1
5	54°39′37.5″	19°63′99.7″	4.0	7.5	0.6	11.6	3.3
6	54°36′62.2″	19°45′00.3″	2.5	7.5	0.6	12.4	2.7
7	54°30′05.6″	19°29′65.3″	2.5	6.9	1.4	12.2	1.1
8	54°28′91.7″	19°42′70.3″	2.4	6.7	0.8	14.7	1.2
9	54°33′17.8″	19°52′02.8″	2.5	7.25	0.6	11.8	2.8

to determine methane in sediments allows recovery of methane at a level of 99% and higher.

Determinations of the methane content were performed on gas chromatography using a flame ionization detector (GC-FID) (Liikanen et al. 2009; Reindl & Bolałek 2014) and a Perkin Elmer Autosystem XL gas chromatograph equipped with a standard non-polar HP-5 capillary column (30 m × 0.32 mm outer diameter; 0.25 mm inner diameter). Helium was used as a carrier gas at a flow rate of 3 $cm^3 min^{-1}$. Chromatographic conditions allowed methane detection at the concentration level of 1 mg kg^{-1} (0.0001%) per sample.

Organic matter content in sediments was determined as loss on ignition (ignition at 550°C, until constant mass was reached). Total carbon was assayed using a Perkin Elmer CHNS/O 2400 elemental analyzer. Organic carbon was assayed after prior removal of carbonates with 1M hydrochloric acid (Hedges & Stern 1984). Black carbon was assayed following the ignition of samples at 375°C for 18 h and their acidification with 1M hydrochloric acid (Elmquist et al. 2004).

Molecular analysis

Techniques for genomic DNA isolation from sediment and PCR amplification of the methanogenic specific gene – the "mcrA gene" (part of the α unit of coenzyme mcr) were used. The techniques were previously described by Innis & Gelfand (1990) with further application by Nunoura et al. (2008) and Steinberg & Regan (2009). General techniques for cloning, described by Sambrook et al. (1989), were applied in our research. Plasmids were automatically sequenced by MacroGen Europe (the Netherlands).

The sequencing results from the clones containing the mcrA gene were later compared using the NCBI BLASTN 2.2.25 application (Mori et al. 2000) containing the data base of previously identified nucleotide sequences of methanogenic Archaea. The similarity between the obtained sequences was explored using this tool, which resulted in the elimination of identical nucleotide sequences. Different DNA sequences of the mcrA gene, obtained from the preliminary selection, are presented in the phylogenetic tree formed using the neighbor-joining method described in detail by Saitou & Nei 1987.

Results

The chemical analysis of the methane content in surface bottom sediments of the Vistula Lagoon proves the presence of this greenhouse gas at all sampling sites (Table 2). The highest methane concentration was found at sites located in the southwestern part of the Vistula Lagoon (sites 7, 8). The lowest methane content was found at sites located in the southeastern part of the basin (sites 3, 4). The total carbon content ranged from 0.16 to 3.75%, organic carbon content was between 0.13 and 2.76%, and black carbon from 0.01 to 0.56%.

Surface sediments collected in the Vistula Lagoon were also the source of genomic DNA containing the specific gene responsible for biological methane production. The comparison of nucleotide sequences of the mcrA gene clones obtained from genomic DNA shows 97% and 90% similarity to the nucleotide sequence of an uncultivated archaeon from the orders *Methanosarcinales* and *Methanomicrobiales* respectively, but most of the genomic DNA isolated from sediments was similar to uncultivated archaea with an unknown metabolic pathway (Fig. 2).

Discussion

The Polish part of the second largest lagoon in the Baltic Sea – the Vistula Lagoon, has never been studied in terms of methane presence, but then methane was found in the Russian part of the Lagoon (Ulyanova et al. 2014). Geological data and sediment characteristics showed that the sediments in this lagoon may potentially feature this greenhouse gas. The intensive inflow of large amounts of debris via the Nogat River

Table 2

Results of chemical analysis of sediment samples from the Vistula Lagoon: total carbon (C_{tot}), organic carbon (C_{org}), black carbon (BC), and methane content ± standard deviation

Site No	C_{tot}	C_{org}	BC	CH_4	
	%			µmol dm^{-3}	
1	3.08	2.49	0.43	12.4	± 2.4
2	2.69	1.85	0.42	23.6	± 1.9
3	2.00	1.32	0.33	9.3	± 2.9
4	2.18	1.38	0.37	7.1	± 2.2
5	2.53	1.99	0.28	25.8	± 2.0
6	0.17	0.13	0.01	14.8	± 2.5
7	2.78	1.81	0.38	6450	± 0.2
8	1.60	1.05	0.32	5140	±0.2
9	3.75	2.75	0.56	36.7	±2.2

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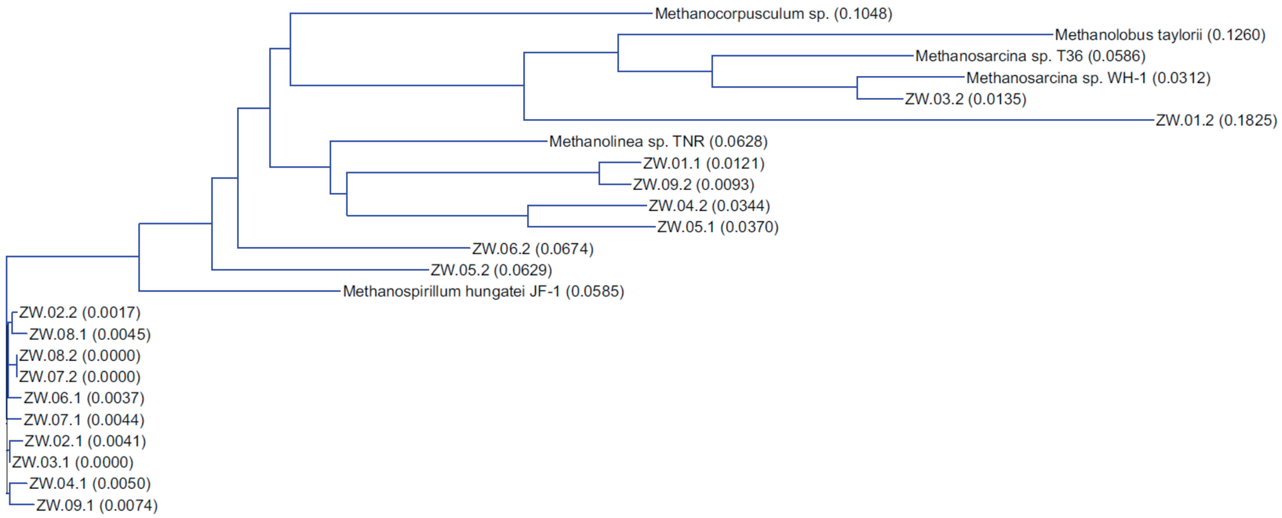

Figure 2

Neighbor-joining phylogenetic tree of wild-type methanogenic clones from the Vistula Lagoon sediments

till 1915 and the continuous inflow of organic matter and biogenic substances via rivers are conducive to primary production (Nieczaj et al. 1975; Wypych et al. 1975; Witek et al. 2010). As a result of primary production, the bottom sediment fraction becomes enriched with organic matter, which may decompose into methane under anaerobic conditions. However, there are no data that would show the presence of a biological factor that could cause its dissimilation in the Polish part of this ecosystem. There are references in the available literature to anaerobic decomposition of algal biomass with hydrogen sulfide production, and oxygen deficiencies as a result of primary production (Nawrocka et al. 2011). Pimenov et al. (2013) indicate methanogenesis in the Russian part of the Vistula Lagoon. The assessment of the functioning of the ecosystem leads to the supposition that organic matter also decomposes into methane as a consequence of the microbiological activity of methanogenic archaea in the Polish part of the lagoon.

In the Russian Part of the Vistula Lagoon, the methane flux from sediments into the water was estimated at 0.45 mmol m^{-2} d^{-1} (Pimenov et al. 2013). Ulyanova et al. (2014) indicate that sediments from the Russian part of the lagoon were rich in methane, which ranged from 17.5 to 528 μmol dm^{-3}. Sediments from the Polish part of the Baltic Sea were already tested for methane. In the sediments from the Gulf of Gdańsk, methane was found within a range of 0.40-4.50 mmol dm^{-3}. Methane was found in sediments from their surface layer down to depths of as much as 100 cm (Brodecka & Bolałek 2011; Brodecka et al. 2013). Similar levels of methane concentration in the sediments of the Baltic Sea were found in the Arkona Basin. Sediments in that part of the Baltic Sea contained from 2.08 mmol dm^{-3} to 4.73 mmol dm^{-3} of methane (Jensen & Fossing 2005). The highest methane concentrations in sediments were found in the Danish Straits, where the maximum concentration reached 8 mmol dm^{-3} at hydrostatic pressure reaching 25 atm (Jørgensen et al. 1990).

The presented research on the bottom sediments of the Vistula Lagoon found the maximum methane concentration in the Elblag region where the water dynamic and salinity are the lowest but the oxygen concentration is relatively high. At sites 7 and 8 (western part), the mean methane concentration in the surface layer of sediments was between 5.14 mmol dm^{-3} and 6.45 mmol dm^{-3}, and these values are close to the maximum methane concentrations in other parts of the southern Baltic Sea. The highest methane concentration was observed at site 7 (west of the lagoon) and was slightly lower than the maximum methane concentration found in Eckernförde Bay (max 6.42 mmol dm^{-3}) or in the aforementioned Arkona Basin (max 4.73 mmol dm^{-3}). At the remaining sites within the Vistula Lagoon, the presence of methane in sediments was found within a range of 0.007 mmol dm^{-3} to 0.037 mmol dm^{-3}. The lowest methane concentrations were determined at sites 3 and 4 in the southeastern part of the basin.

It is estimated that the largest source of methane in the marine environment is the microbiological decomposition of organic matter under anaerobic conditions. Methane in seas and oceans may result from thermogenic changes of fossil organic matter

(Edlund 2007; Reeburgh 2007). The purpose of this study, however, was not to estimate the proportions of particular sources of methane in sediments, but rather to determine the presence of a biological factor responsible for the production of this greenhouse gas.

On the basis of the molecular testing of the Vistula Lagoon surface sediments, it was confirmed that a biological factor is present (Fig. 2). The obtained nucleotide sequences indicated the highest similitude to the sequences of methanogenic archaea of the *Methanomicrobiales* and *Methanosarcinales* orders. Previous studies of the water in Puck Bay proved the presence of sequences of methanogenic archaea of the *Methanosarcinaceae*, *Methanospirillaceae* and *Methanocorpusculaceae* families (Reindl & Bolałek 2012b). Both Puck Bay and the Vistula Lagoon are classified as border waters of the lagoon type. However, the studies by Brodecka & Bolałek (2011) also confirmed the presence of methane in the open waters of the Baltic Sea – the Gdańsk Deep.

The analysis of three sediment samples collected in the area covered by Brodecka & Bolałek's studies (2011) confirmed the presence of the mcrA gene in the genomic DNA isolated from the sediments of this area in the Baltic Sea. A comparison of nucleotide sequences collected from lagoon areas with those from the open sea is presented in Figure 3. Most of the obtained nucleotide sequences represented arrangements of base pairs that do not show a significant resemblance to known and described microorganisms. Therefore, these basins have a considerable potential in terms of the microbiological diversity of organisms inhabiting their sediments. This is also related to potentially diverse alimentary preferences. In conclusion, these studies confirmed that methane-forming bacteria participated in the production of methane found both in the sediments of the Vistula Lagoon and in the Gdańsk Deep.

Conclusions

The study of methane in organic-rich sediments of the Vistula Lagoon indicates its presence at all sites located in the Polish part of the lagoon. The concentration of methane at a relatively high level,

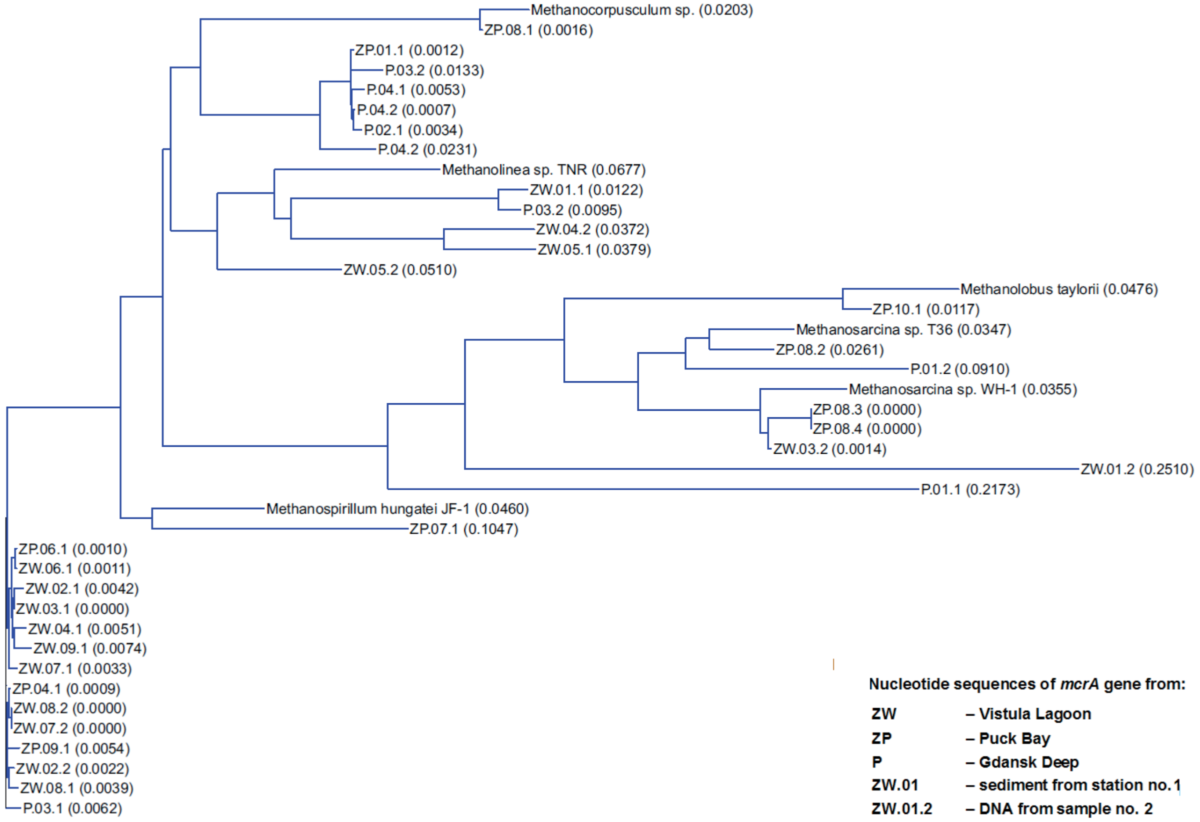

Figure 3

Neighbor-joining phylogenetic tree of wild-type methanogenic clones from the Vistula Lagoon, Puck Bay and Gdańsk Deep sediments which might be near saturation values, suggested gas bubbles in the sediments. Such situation was observed in the coastal area of Puck Bay (Reindl & Bolałek 2012a). Eutrophication provides organic matter to the sediment, which has a significant impact on biological methane production (Bange et al. 1994; Bange 2006). Previous studies of a similar ecosystem (Puck Bay) confirm the presence of DNA belonging to methanogenic archaea in the sediments enriched with organic matter (Reindl & Bolałek 2012b). Similarly to other areas of the southern Baltic Sea, organic matter in the Vistula Lagoon was decomposed under anoxic conditions within a biological factor, which results in methane production in the sediments.

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