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Microbial methane oxidation at the redoxcline of the Gotland Deep (Central Baltic Sea)

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Abstract

Methane concentrations in the stratified water column of the Gotland Deep (Central Baltic Sea) show a strong gradient from high values in the saline deep water (max. 504 nM) to low concentrations in the less dense, brackish surface water (about 4 nM). The steepest gradient is present within the redoxcline (between 115 and 135 m water depth) that separates the anoxic deep part from the oxygenated surface water, implying a methane consumption rate of 0.28 nM d^{-1}. The process of microbial methane oxidation within the redoxcline is mirrored by a shift of the stable carbon isotope ratio of methane between the bottom water ($\delta^{13}C$ CH_4 = −82.4 ‰) and the suboxic depth interval ($\delta^{13}C$ CH_4 = −38.7 ‰). A water column sample from 100 m water depth was studied to identify the microorganisms responsible for the methane turnover at the redoxcline. Notably, methane monoxygenase gene expression analyses for the specific water depth demonstrated that accordant methanotrophic activity was due to only one microbial phylotype. An imprint of these organisms on the particular organic matter was revealed by distinctive lipid biomarkers showing bacteriohopanepolyols and lipid fatty acids characteristic for aerobic type I methanotrophic bacteria (e.g. 35-aminobacteriohopane-30,31,32,33,34-pentol). In conjunction with earlier findings, our results support the idea that biogeochemical cycles in Central Baltic Sea redoxclines are mainly driven by only a few microbial key species.

1 Introduction

Methane as an atmospheric trace gas is known to have a relevant impact on Earth's climate. Although aquatic systems represent the most significant source of atmospheric methane, the importance of the marine system seems to be marginal (Bange et al., 1994), although enormous amounts of methane are formed in marine sediments (Reeburgh, 2007). One effective mechanism that is limiting the flux of methane from the sedimentary reservoir into the atmosphere is the microbial oxidation of methane in the

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sediment and the water column (Reeburgh, 2007). Comprehensive studies on aquatic sediments in different settings show that methane is microbially oxidized by the use of different electron acceptors like oxygen, sulfate, iron, manganese and nitrite (Hinrichs and Boetius, 2002; Reeburgh, 2007; Beal et al., 2009; Ettwig et al., 2010). Although these processes are efficient and consume the main part of dissolved methane before it escapes at the sediment/water interface, some parts of the ocean are characterized by strongly elevated methane concentrations in the water column. This holds particularly true for stagnant, oxygen-deficient basins like the Black Sea, Cariaco Basin or Central Baltic Sea (Scranton et al., 1993; Kessler et al., 2006; Schmale et al., 2010a). Compared to the number of studies on the microbial processes of methane oxidation in sediments, water column studies are scarce, and could to date just identify the oxidation of methane through oxygen and sulfate (Reeburgh, 2007). Nevertheless, multidisciplinary studies in the water column of the Black Sea could impressively demonstrated that the flux of methane from the deep-water reservoir into the atmosphere is effectively buffered by the microbial oxidation of methane under anaerobic and aerobic conditions (Schubert et al., 2006; Blumenberg et al., 2007; Schmale et al., 2011).

Our present investigations were carried out in the Gotland Deep in the central part of the Baltic Sea (Fig. 1). The Baltic Sea is a European semi-enclosed marginal sea characterized by limnic to brackish surface water and more saline deep and bottom water. Especially for the central deep basins of the Baltic Sea, this results in limited vertical mixing, the development of a prominent redoxcline with oxic to anoxic conditions, and the formation of stable biogeochemical zones (Nausch et al., 2008). In these basins, the stagnant deep water can only be renewed by strong temporal inflow events of saline oxygenated water from the North Sea (Reissmann et al., 2009) or by long term vertical transport mechanisms mainly induced by bottom boundary mixing along the sloping topography (Holtermann and Umlauf, 2012). More frequent are weak inflows of North Sea water that are periodically perturbing the intermediate water column stratification and biogeochemical zones in the central basins (Matthäus et al., 2008). The Baltic Sea, like other marginal seas, is characterized by high terrestrial inputs and

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production rates of organic matter that are to a considerable extent accumulated and decomposed in the sediment. Under anoxic conditions, the final step of decomposition of organic matter leads to the generation of methane within the sediment. In the Baltic Sea, pore-water as well as acoustic investigations demonstrated that methane is abundant in high concentrations within the sediment and that in some regions methane is also released as free or dissolved gas into the water column (Dando et al., 1994; Piker et al., 1998; Thießen et al., 2006). Extensive water column investigations in the Baltic Sea identified the strongest methane enrichment within the stagnant anoxic water bodies of the deep basins (Gotland Deep and Landsort Deep; max. 504 nM at 230 m water depth and 1058 nM at 435 m water depth, respectively; Schmale et al., 2010b). In contrast, surface water methane concentrations in these areas are only slightly enriched compared to the atmospheric equilibrium, indicating an effective sink that prevents the escape of methane from the deep water into the atmosphere (Schmale et al., 2010b). However, little is as yet known about the processes that regulate the methane flux in these environments. In this paper, we present data on the methane sink mechanism in the water column of the Gotland Deep. Applying a multidisciplinary approach that combines gas chemistry, molecular biology and lipid biomarker geochemistry, we focus on the processes at the oxic/anoxic transition zone (redoxcline) that is periodically perturbed by intrusions triggered by inflows of North Sea waters.

2 Methods

Samples were retrieved during a scientific cruise in summer 2008 with the German R/V *Maria S. Merian* (MSM 08/3, 18 June to 18 July). The Gotland Deep (57° 18′ N and 20° 04′ E; Fig. 1) represents the deepest location in the Eastern Gotland Basin (water depth at our water station 231 m). The sampling strategy at this location was directed at (1) identifying the depth interval of methane oxidation based on physical parameters and on board gas chemistry, and (2) recovering samples from the relevant depth

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interval for home-based molecular biological and lipid biomarker studies to identify the microorganisms involved in methane oxidation.

2.1 Physical parameters and gas chemistry

Water stations for analyses of the gas chemistry were carried out with a rosette water sampler equipped with twenty-four 10 l Hydrobios-Freeflow bottles. For continuous CTD and turbidity profiling a Seabird sbe911+ system, together with a turbidity sensor (ECO FLNTU, WET Labs) were attached to the underwater unit.

The oxygen distribution was measured according to Winkler's method, whereas hydrogen sulfide was analysed colorimetrically with the methylene blue method (Grasshoff, 1983).

Water samples (600 ml) for methane analyses were transferred directly from the sample bottle into pre-evacuated 1100 ml glass bottles. Dissolved methane was extracted using a vacuum degassing method and its mole fraction was determined with a gas chromatograph equipped with a flame ionization detector (Trace GC, Thermo Electron). The average precision of this method is ±3 % (Keir et al., 2009).

For the determination of $\delta^{13}C$ CH_4 values, subsamples of the extracted gas were analyzed at the Leibniz Institute for Baltic Sea Research Warnemünde using an isotope-ratio mass spectrometer (modified after Schmale et al., 2010a). These subsamples were collected in 10 ml pre-evacuated crimp-top glass vials containing 4 ml of super-saturated salt solution (degassed Millipore water, poisoned with $HgCl_2$) and sealed with a butyl rubber septum. Stable carbon isotope analysis involved removal of water and carbon dioxide on a NaOH/Ascarite trap, double cryofocussing at −110 °C (ethanol/nitrogen) on Hayesep D and Poraplot S columns, gas-release by heating the traps separately to 40 °C and gas separation on a MolSieve 5Å Plot capillary column (Supelco, 30 m, I.D. 0.32 mm) at 30 °C (Trace GC Ultra, Thermo Electron), combustion to CO_2 using a Ni catalyst at 1050 °C, removal of combustion water using a Nafion trap, and injection into a MAT 253 mass spectrometer (Thermo Electron, Bremen) using a continuous flow technique. The $\delta^{13}C$ CH_4 data is expressed vs. Vienna Pee

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Dee Belemnite (VPDB) standard. Calibration of the system was performed daily by the use of a CH_4 standard with known isotopic composition. The average precision of that method is ±1 ‰.

2.2 Lipid biomarkers

214 l of water from 100 m water depth were filtered on glass microfiber filters (ø 30 cm; 0.7 µm pore size) using a PUMP-CTD system (Strady et al., 2008). Half of the filter was extracted in triplicate with dichloromethane and methanol (3 : 1, v : v) in a CEM Mars 5 microwave (Matthews, NC) at 80 °C and 800 W. An aliquot of the sample was acetylated with acetic acid/pyridine as described elsewhere (Blumenberg et al., 2007) and analysed using high performance liquid chromatography-mass spectrometry (LC-MS). LC-MS was performed using a Varian Prostar Dynamax HPLC system coupled to a Varian 1200 l triple quadrupole mass spectrometer (for analytical details see Blumenberg et al., 2010). Another aliquot of the extract was separated by column chromatography into a hydrocarbon (F1), an alcohol and ketone (F2), and a polar fraction (F3) using a column (ø ∼1 cm) filled with 7.5 g silica gel 60 (according to Blumenberg et al., 2010). F3 was transmethylated using trimethylchlorosilane in methanol (1 : 8; v : v; 1.5 h at 80 °C). Double bond positions within unsaturated fatty acid methyl esters were determined by derivatisation with dimethyldisulfide (DMDS; method modified after Carlson et al., 1989 and Gatellier et al., 1993). The polar fraction (F3), and the DMDS derivatized sample were analyzed with coupled gas chromatography-mass spectrometry (GC-MS) using a Varian CP-3800 gas chromatograph equipped with a fused silica column (Phenomenex Zebron ZB-5MS, 30 m, I.D. 0.32 mm) coupled to a Varian 1200 l mass spectrometer. He was used as carrier gas. The temperature program was 80 °C (3 min) to 310 °C (held 25 min) at 4 °C min^{-1}. Compounds were identified by comparing mass spectra and retention times to published data. $\delta^{13}C$ values of fatty acid methyl esters from the polar fraction (F3) were measured in replicate as described previously (Blumenberg et al., 2010).

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2.3 *pmoA* gene expression analyses

An additional filter sample was taken in 100 m water depth using the rosette water sampler. 1000 ml sample water were filtered on a Durapore filter (0.2 µm pore size), frozen in liquid nitrogen and stored at −80 °C.

RNA was extracted from the frozen filter with acidic phenol (Weinbauer et al., 2002) and quantified using a NanoDrop ND-1000 spectrometer (NanoDrop Technologies). To generate *pmoA*-specific cDNA 100 ng RNA were reverse transcribed using iScript Select cDNA Synthesis Kit (Biorad) and reverse primer mb661r (Costello and Lidstrom, 1999). To check for potential DNA contamination one sample was incubated without reverse transcriptase. 1 µl of cDNA was amplified by polymerase chain reaction (PCR). For the generation of specific GC-clamped PCR products a discontinuous PCR was applied: reactions (50 µl) containing 1 × PCR buffer, 200 µM of each dNTP, 0.3 µM revere primer mb661r, 0.1 µM forward primer A189f (Holmes et al., 1995), 0.5 mM $MgCl_2$, 0.5 µl polymerase (Herculase II, Fusion) and template cDNA were incubated at an initial 94 °C for 5 min and after 20 cycles of 60 s at 94 °C, 60 s at 56 °C and 30 s at 72 °C the PCR was paused at 72 °C and 0.12 µM A189f_GC primer were added to each reaction. Afterwards the PCR was resumed for another 15 cycles with conditions as described above, followed by a final elongation step of 5 min at 72 °C. Specificity of the PCR products was documented by agarose gel electrophoresis and staining with ethidium bromide. The described discontinuous PCR yielded more specific and distinct PCR products than a conventional PCR with GC-primer (data not shown).

PCR products were further analysed by Denaturing Gradient Gel Electrophoresis (DGGE) with a gradient of 35 % to 80 % denaturant in a 6 % polyacrylamide gel. Electrophoresis ran at 100 V and 60 °C for 16 h in 1 × TAE buffer. The gel was stained with a 1 : 5000 dilution of SYBRGold (Invitrogen) for 30 min. Bands were excised and reamplified in a PCR reaction containing 1 × PCR buffer, 0.3 µM of A189f and mb661r each, 200 µM of each dNTP and 0.5 µl polymerase in 30 cycles with an annealing temperature of 56 °C. PCR products were purified with NucleoSpin purification kit

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(Macherey-Nagel) and sequenced with primers A189f and mb661r by AGOWA (Berlin, Germany).

Forward and reverse sequences were checked for quality applying Seqman software (DNASTAR).

For phylogenetic analysis the ARB software package was used (Ludwig et al., 2004). Alignment was based on partial DNA sequences of *pmoA* and *amoA* genes obtained from GenBank Database with partial sequences of *amoA* serving as an outgroup in the tree construction (Database accession numbers: AF037107, AF043710, AF037108). Sequences for analysis were reduced to unambiguously alignable positions.

Three different trees were calculated using the algorithms maximum likelihood (PHYML), maximum parsimony and neighbor-joining with Jukes-Cantor correction.

Nucleotide sequence accession numbers is deposited in the GenBank database (accession number will be provided after acceptance of the manuscript).

3 Results and discussion

3.1 Physical parameters and gas chemistry

The estuarine circulation in the Baltic Sea causes a strong vertical salinity gradient between the surface and deep water (Lass and Matthäus, 2008). This gradient is very pronounced in the deep basins of the Central Baltic Sea (e.g. Gotland and Landsort Deep; Fig. 2) and reflects a water column stratification that limits the vertical mixing and water renewal in the deep strata (Reissmann et al., 2009). During summer, mixing processes are further inhibited by a temperature gradient between the warm surface water and the cold intermediated winter water (Fig. 2, gradient between 15–25 m water depth). The homogeneous temperature distribution within the upper 15 m during sampling indicates a well mixed surface water layer (Fig. 2).

During sampling, the specific water column structure led to oxygen deficiency below a water depth of about 90 m. Further downward, the oxygen concentrations decreased

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below 0.2 ml l^{-1}, characterizing the suboxic depth interval between the oxic surface and anoxic deep waters (Nausch et al., 2008). The lower boundary of the suboxic layer was located at about 138 m water depth where the concentration of hydrogen sulphide (H_2S) started to increase. A distinct turbidity anomaly was observed at about 120 m water depth (Fig. 2). This specific feature is known from other anoxic basins like the Black Sea and is most likely caused by the precipitation of iron and manganese oxides (Kempe et al., 1991) and an enrichment of particulate organic matter (POM) due to enhanced microbial activity (Prokhorenko et al., 1994). The concentrations of H_2S and other reduced chemical species like ammonium (NH_4^+) are constantly increasing with depth indicating an upward flux from the sediment or deep water towards the redoxcline (Nausch et al., 2008). The same concentration pattern was observed for methane (Fig. 2). Highest methane concentrations were detected close to the seafloor (504 nM at 230 m water depth) supporting an origin from methanogenesis in the sediment (Piker et al., 1998). Indeed, low $\delta^{13}C$ CH_4 values (−82.4 ‰ to −75.2 ‰, Fig. 2) observed in the anoxic water body clearly point at a microbial methane source (Whiticar, 1999). The methane concentration profile shows a pronounced decrease within the suboxic zone from 124 nM at 135 m water depth to 4.8 nM at 115 m water depth. At the same time, $\delta^{13}C$ CH_4 values substantially increase (up to −38.7 ‰ at 80 m water depth). As microbial reactions favour the incorporation of ^{12}C and thus, depletion of $^{12}CH_4$ in the residual methane pool, this isotopic shift clearly indicates microbial methane oxidation within that water level (Whiticar, 1999). In a first approximation the methane oxidation rate can be derived from the methane gradient and the vertical transport velocity. Using the vertical diffusivity (k_z) of 0.95 $m^2 d^{-1}$ (Axell, 1998) in combination with the methane distribution between 115 m (4.8 nM) and at 135 m water depth (124 nM) this calculation leads to a flux of methane of 5.7 µmol $m^{-2} d^{-1}$. If we assume that this flux is oxidized within the 20 m depth interval, we receive a methane consumption rate of 0.28 nM d^{-1}. An inverse trend of the methane carbon isotope ratios is observed above the suboxic layer (Fig. 2; $\delta^{13}C$ ratios between −59.9 ‰ and −48.5 ‰). This trend is probably caused by (1) the downward ventilation of atmospheric methane (−47.4 ‰;

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http://www.esrl.noaa.gov/gmd/ccgg/iadv/), and/or (2) microbial methane production in shallow waters. The process of methane formation in an oxygenated water column has been observed in many regions (Holmes et al., 2000; Schmale et al., 2010a) and seems to be related to the decay of methylphosphonates, in particular under phosphate-limiting conditions, and/or methanogenesis in the anoxic interior of particles (Karl et al., 2008). Such methane forming processes are also indicated in our dataset by a pronounced $^{12}CH_4$ enrichment at 20 m water depth ($\delta^{13}C = -59.9‰$) together with slightly elevated methane concentrations of 7 nM (surrounding water depths around 4 nM). However, within the surface water, methane is only slightly enriched compared with the atmospheric equilibrium (144 % saturation ratio; Schmale et al., 2010b), indicating that the local emission of methane into the atmosphere is rather low.

3.2 Methanotrophic microorganisms at the redoxcline

Chemical gradients feature versatile environments and are known to harbour enhanced microbial abundance and activity. In the redoxcline of the Central Baltic Sea, various biogeochemical processes have been identified, such as denitrification, ammonia oxidation, or dark CO_2 fixation (Labrenz et al., 2005; Jost et al., 2008; Glaubitz et al., 2009; Labrenz et al., 2010). However, nothing is known about microbial methane oxidation in this setting.

To gain information on the contribution of methanotrophic microorganisms to the POM at the redoxcline in 100 m water depth, we studied concentrations and distributions of bacteriohopanepolyols (BHPs) and fatty acid biomarkers, and performed expression analyses of the methane monooxygenase gene (*pmoA*).

Of special biomarker value are BHPs with an A-ring methylation at C-3 (Neunlist and Rohmer, 1985) and/or an amino group at C-35 of the hopanoid structure, both of which are widespread in methanotrophic bacteria (Neunlist and Rohmer, 1985; Talbot et al., 2001). The vast majority of BHPs in 100 m water depth of the Gotland Deep was composed of bacteriohopane-32,33,34,35-tetrol (BHT) and 35-aminobacteriohopane-32,33,34-triol (aminotriol), the most common and thus

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unspecific BHPs (Fig. 3). C-3 methylated BHPs were not observed. However, low abundances of 35-aminobacteriohopane-31,32,33,34-tetrol (aminotetrol) and of 35-aminobacteriohopane-30,31,32,33,34-pentol (aminopentol) were found (Fig. 3). Whereas these both amino-BHPs are considered indicative of methanotrophic bacteria (Neunlist and Rohmer, 1985; Talbot and Farrimond, 2007), particularly the latter is even regarded as a biomarker for the type I subgroup (gamma proteobacteria; Talbot and Farrimond, 2007). Further evidence for a prominent contribution of type I methanotrophs comes from the fatty acids C16:1ω8c and C16:1ω5c which are considered as specific to this group (Makula, 1978; Nichols et al., 1985; Table 1). At the same time the lack of C18:1ω8c, a fatty acid specific of type II methanotrophs (alpha proteobacteria, Bowmann et al., 1991), indicates that these microorganisms do not play a significant role for the methane turnover at the redoxcline of the Gotland Deep. Biomarkers from methanotrophic bacteria commonly mirror the isotopic traits of the substrate (Summons et al., 1994). Indeed the δ^{13}C values of the fatty acids C16:1ω8c and C16:1ω5c (−38.8 ‰ and −35.5 ‰, respectively; Table 1) are well within the δ^{13}C CH_4 at 80 and 105 m (=−38.7 ‰ and −50.6 ‰, respectively; Fig. 2). Whereas robust biomarker indications for the presence of methanotrophic bacteria exist, their relative abundance among the bacterial community appears to be low. This is indicated (i) by the low proportion of methanotroph-specific amino-BHPs within the total BHPs (<1.6 % of total BHPs; note that amino-BHPs are often predominant in methanotrophs; Talbot et al., 2001), and (ii) by the low amounts of type I specific fatty acids acids (C16:1ω8c and C16:1ω5c represent 1.4 % of total fatty acids).

The presence of methanotrophic bacteria was further supported by molecular biological studies carried out on a sample from the same water depth. Although the two groups of methanotrophs, type I and type II, use different physiological pathways for the assimilation of carbon from methane, namely the ribulose monophosphate pathway and the serine pathway, the key enzyme methane monooxygenase responsible for the initial oxidation of methane to methanol is present in both groups. The gene coding for the alpha subunit of the particulate form of the enzyme (*pmoA*) has been used

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as a marker for the detection and characterization of methanotrophic communities in different habitats (Costello and Lidstrom, 1999; Bourne et al., 2001; Chen et al., 2007, 2008). In order to identify active methanotrophs we investigated *pmoA* gene expression in situ. Based on DGGE analysis only one type of *pmoA* transcript was present, phylogenetically affiliated with the type I methanotrophs and most similar to an uncultured bacterium found in a meromictic crater lake (Fig. 4). Thus, in contrast to studies in the redoxcline of the Black Sea, where indications for type I, II and X were found (Gal'chenko et al., 1988; Durisch-Kaiser et al., 2005; Blumenberg et al., 2007), the diversity of active methanotrophs in the Gotland Deep seems to be lower. Although our current finding is based on one sampling depth, it is in line with previous studies investigating the microbial catalysts of denitrification, nitrification, or dark CO_2 fixation in Central Baltic Sea redoxclines which also revealed that these pathways were driven by only a few bacterial or archaeal key species (Grote et al., 2008; Glaubitz et al., 2009; Labrenz et al., 2010). An explanation for the reduced microbial diversity along the Central Baltic Sea redoxclines could be the periodic perturbation of the stratification which does not occur in the same strength and frequency in the Black Sea. An overlap of sulfide- and oxygen-containing waters can occur in the Gotland Basin (Axell, 1998), and it is known that sulfide is toxic for many organisms or at least can inhibit the activity of specific microorganisms (Erguder et al., 2009). Thus, an advanced diversification of methanotrophic bacteria could have been inhibited by sulfide stress and, possibly, outcompetes other than type I methanotrophic bacteria at the redoxcline of the Central Baltic Sea.

4 Conclusions

Using a multidisciplinary approach of gas chemistry, lipid geochemistry, and molecular biology, we identified the process of aerobic methane oxidation within the pelagic redoxcline of the Gotland Deep (Central Baltic Sea). This was evidenced by a strong decrease in methane concentrations together with a ^{13}C CH_4 enrichment, the occurrence

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of lipids specific for methanotrophic bacteria (e.g. aminopentol; 16:1ω8c fatty acid), and the detection of the key enzyme methane monooxygenase (*pmoA*). Phylogenetic and biomarker data retrieved from a 100 m water column sample demonstrate that the diversity of active methanotrophs in the Gotland Deep is restricted to members of the type I subgroup. In contrast to other marine settings with a permanent stratification, e.g. the Black Sea, the physical and biogeochemical structure of the Gotland Deep is periodically disturbed by inflows or long term vertical transport mechanisms. How this is affecting the methane turnover in the water column and the microbial community responsible for this process is a fascinating question that needs to be investigated in future studies.

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Table 1. Concentrations, relative abundances and $\delta^{13}C$ values of individual fatty acids (analyzed as methyl ester derivatives) at 100 m water depth of the Gotland Deep. Fatty acids specific for methanotrophic bacteria are given in bold letters.

Fatty acid	Concentration ($\mu g\,g\,C_{org}^{-1}$)	% of total fatty acids	$\delta^{13}C$ (‰)
C14:0	3.78	0.7	−26.9
iC15:0	8.61	1.6	−21.5
aiC15:0	10.14	1.9	−26.2
C15:0	8.6	1.6	−25.8
iC16:0	2.9	0.5	−29.7
C16:1ω9t	3.1	0.6	−22.2
C16:1ω8c	1.0	0.2	**−38.8**
C16:1ω8t	3.3	0.6	−30.4
C16:1ω7c	23.1	4.2	−27.6
C16:1ω7t	5.7	1.0	–
C16:1ω5c	6.6	1.2	**−35.7**
C16:1ω5t	2.2	0.4	−33.8
C16:0	130.0	23.7	−26.9
iC17:0	1.0	0.2	−29.6
aiC17:0	1.6	0.3	−28.6
C17:0	6.3	1.2	−30.9
C18:2	4.1	0.8	−25.4
C18:3	3.3	0.6	–
C18:1ω9c	24.6	4.5	−26.5
C18:1ω7c	23.3	4.2	−24.9
C18:1ω6c	1.5	0.3	−30.9
C18:1ω5c	0.9	0.2	−20.2
C18:0	227.9	41.6	−27.1
iC19:0	45.0	0.9	−26.5
C19:0	3.8	0.7	–
C20:0	15.4	2.8	−29.6
C21:0	2.0	0.4	–
C22:0	10.3	1.9	−29.7
C24:0	7.7	1.4	–

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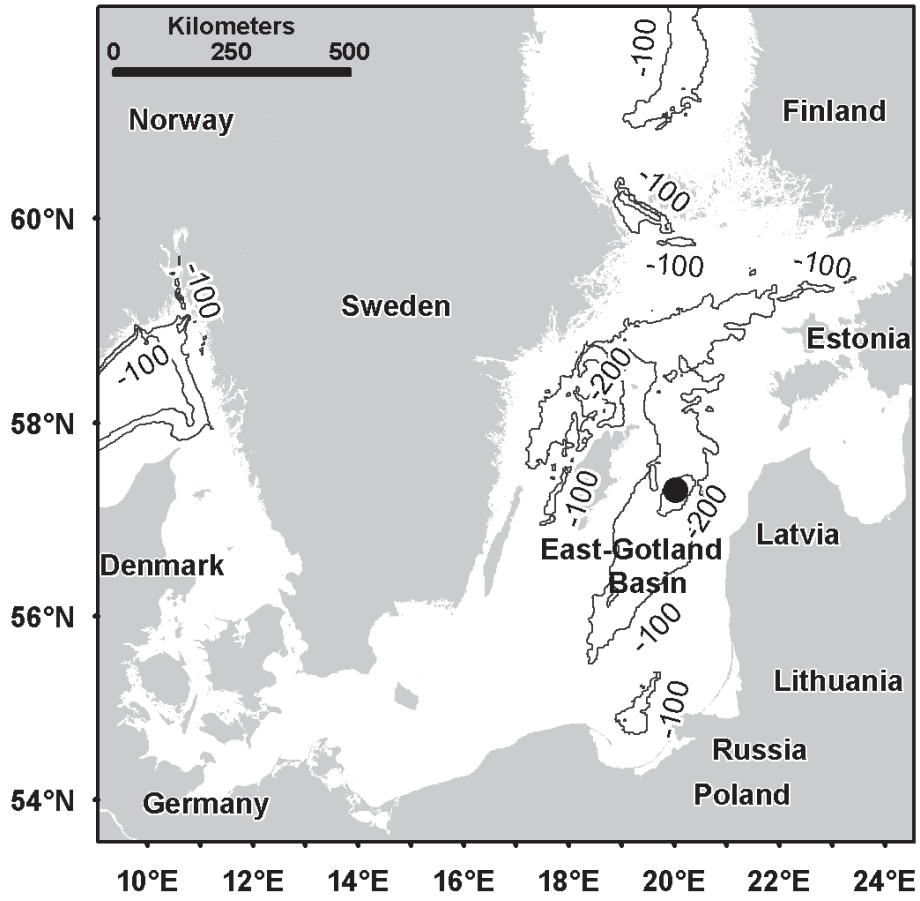

Fig. 1. The Baltic Sea and the location of the Gotland Deep. The study area is indicated with a black dot.

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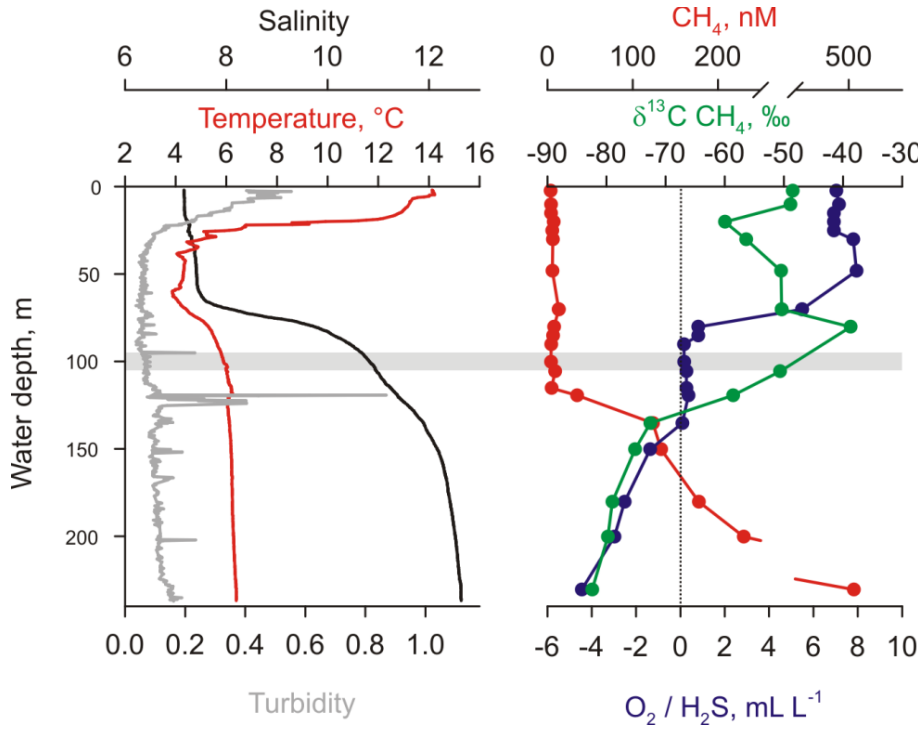

Fig. 2. Left: vertical distribution of salinity (black), temperature (red), and turbidity (gray). Right: vertical distribution of oxygen and hydrogen sulfide (expressed as negative oxygen equivalents, blue), methane (red), and $\delta^{13}C$ value of methane (green). The water depth for filter samples (100 m) is indicated with a gray shaded bar.

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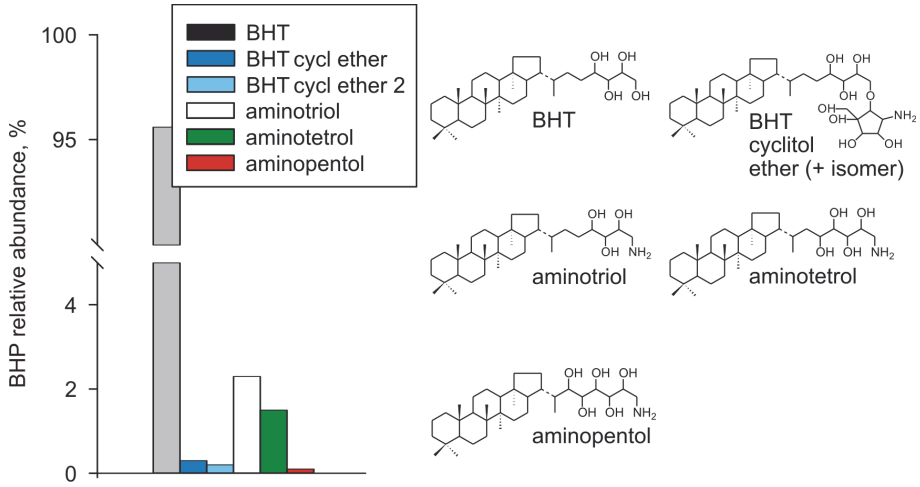

Fig. 3. The relative abundances of specific bacteriohopanepolyols (BHPs) sampled in 100 m water depth, together with the chemical structure of each compound. BHT = bacteriohopantetrol; cycl = cyclitol.

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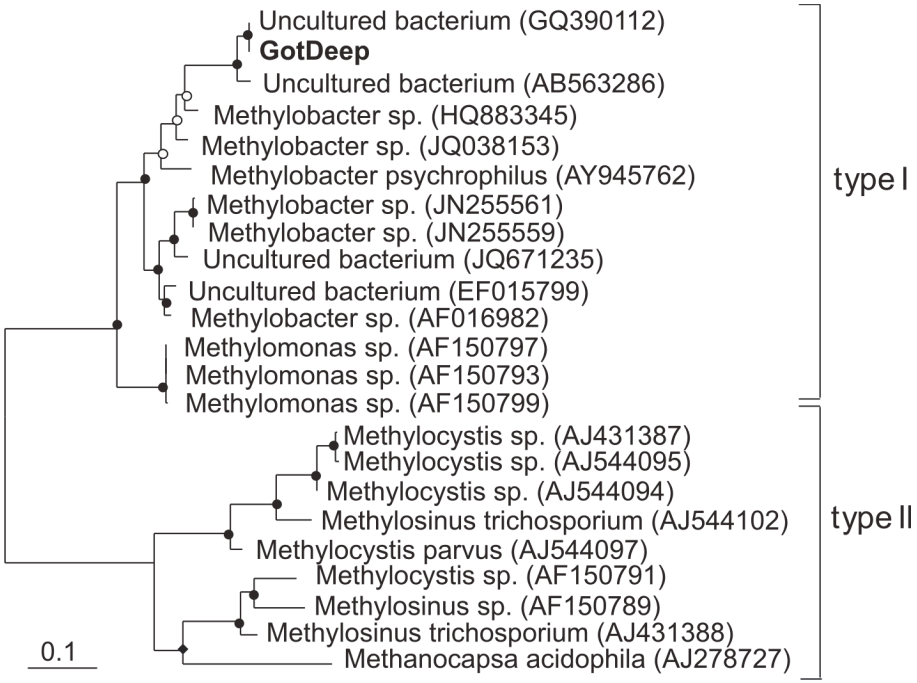

Fig. 4. Unrooted maximum likelihood tree showing the phylogenetic affiliation of the partial *pmoA* DNA sequence generated from the filter sample taken in 100 m water depth (marked bold). Black circles = validation of subtree by neighbor-joining and parsimony; white circles = validation of subtree by parsimony; black diamond = validation of subtree by neighbor-joining. Scale bar represents 10 substitutions per 100 nucleotides. For tree construction partial *amoA* sequences were used as an outgroup (not shown).

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