Biomarkers in the stratified water column of the Landsort Deep (Baltic Sea)

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Abstract

The water column of the Landsort Deep, central Baltic Sea, is stratified into an oxic, suboxic and anoxic zone. This stratification controls the distributions of individual microbial communities and biogeochemical processes. In summer 2011, particulate organic matter was filtered from these zones using an *in situ* pump. Lipid biomarkers were extracted from the filters to establish water-column profiles of individual hydrocarbons, alcohols, phospholipid fatty acids, and bacteriohopanepolyols (BHPs). As a reference, a cyanobacterial bloom sampled in summer 2012 in the central Baltic Sea Gotland Deep was analyzed for BHPs. The biomarker data from the surface layer of the oxic zone showed major inputs from cyanobacteria, dinoflagellates and ciliates, while the underlying cold winter water layer was characterized by a low diversity and abundance of organisms, with copepods as a major group. The suboxic zone supported bacterivorous ciliates, type I aerobic methanotrophic bacteria, sulfate reducing bacteria, and, most likely, methanogenic archaea. In the anoxic zone, sulfate reducers and archaea were the dominating microorganisms as indicated by the presence of distinctive branched fatty acids, archaeol and PMI derivatives, respectively. Our study of *in situ* biomarkers in the Landsort Deep thus provided an integrated insight into the distribution of relevant compounds and describes useful tracers to reconstruct stratified water columns in the geological record.

1 Introduction

The Baltic Sea is a brackish marine marginal Sea with a maximum depth of 459 m in the Landsort Deep (western central Baltic Sea; Matthäus and Schinke, 1999; Reissmann et al., 2009; Fig. 1). A positive freshwater budget and saltwater inflows from the North Sea through Skagerrak and Kattegat lead to a permanent halocline that stratifies the water column of the central Baltic Sea at about 60 m water depth (Reissmann et al., 2009). Major saltwater inflows, as detected in 1993 and 2003, sporadically disturb the stratification in the eastern central Baltic Sea and oxygenate the suboxic zone and deep water. These inflows, however, rarely reach the western central Baltic Sea. Even the strong inflow from 1993 had only minor effects on Landsort Deep, where stagnating conditions prevailed throughout (Bergström and Matthäus, 1996). Therefore, the Landsort Deep offers stable environments for microbial life within the oxic, suboxic and anoxic zones, and provides an excellent study site for the investigation of biomarker inventories that specify stratified water columns.

The Black Sea, although much larger in size, is comparable with the Landsort Deep with respect to the existence of a permanently anoxic deep water body. Two comprehensive *in situ* biomarker reports gave a wide-ranging overview of various biomarkers and their producers in the Black Sea water column, and identified a close coupling of microorganisms to biogeochemically defined water layers (Wakeham et al., 2007; 2012). Several other *in situ* biomarker water-column studies exist, but were usually focused on certain aspects, for example anaerobic and aerobic methanotrophy (Schouten et al., 2001; Schubert et al., 2006; Blumenberg et al., 2007; Sáenz et al., 2011; Xie et al., 2014, and others).

For the Baltic Sea water column, biomarker knowledge is limited as most studies so far were focused on pollution related compounds (e.g. Beliaeff and Burgeot, 2001; Lehtonen et al., 2006; Hanson et al., 2009). Recently, we reported the water-column distributions and ^{13}C-content of individual bacteriohopanepolyols (BHPs) and phospholipid fatty acids (PLFA) from the Gotland Deep, located about 150 km SE of the Landsort Deep in the eastern central Baltic Sea. These studies were aimed at microbial methane turnover and confirmed the importance of the Baltic Sea suboxic zone for bacterial methane oxidation (Schmale et al., 2012; Berndmeyer et al., 2013; Jakobs et al., 2014). The theoretical possibility of sulfate-dependent methane oxidation in the anoxic zone was also stated (Jakobs et al., 2014), but still remains to be proven for the central Baltic Sea water column.

Because the eastern central Baltic Sea is regularly disturbed by lateral intrusions in intermediate water depths (Jakobs et al., 2013), we chose the more stable Landsort Deep in the western central Baltic Sea as a sampling site for this biomarker study. Furthermore,

published genetic studies reporting on prokaryotes and the related metabolisms in the water column of the Landsort Deep (Labrenz et al., 2007; Thureborn et al., 2013) provide a background to which the organic geochemical results can be advantageously related. The depth profiles of biomarkers from this setting not only reveal how actual biogeochemical processes are reflected by lipid abundances, distributions and stable carbon isotope signatures, they also provide reference data for the reconstruction of past water columns using biomarkers from the sedimentary record.

2 Material and methods

2.1 Samples

Samples were taken during cruise 06EZ/11/05 of R/V *Elisabeth Mann Borghese* in summer 2011. The Landsort Deep is located north of Gotland (58°35.0' N 18°14.0' E; Fig. 1). A Seabird sbe911+ CTD system and a turbidity sensor ECO FLNTU (WET Labs) were used for continuous water-column profiling. Oxygen and hydrogen sulfide concentrations were measured with Winkler's method and colometrically, respectively (Grasshoff et al., 1983). Filter samples of 65 to 195 L obtained from 10, 65, 70, 80, 90, 95 and 420 m water depth were taken with an *in situ* pump and particulate material was filtered onto precombusted glass microfiber filters (Ø 30cm; 0.7 µm pore size; Munktell & Filtrak GmbH, Germany). Filters were freeze dried and kept frozen at -20° C until analysis.

A cyanobacterial bloom was sampled in summer 2012 on cruise M87/4 of R/V *Meteor* at the Gotland Deep (57°19.2'N, 20°03.0'E; Fig. 1), east of Gotland. Water samples of 10 L were taken at 1 m water depth and filtered with a 20 µm net. The samples were centrifuged and the residue freeze dried. Samples were kept frozen at -20° C until analysis.

2.2 Bulk CNS analysis

Three pieces (Ø 1.2 cm) from different zones of the filters were combusted together with V_2O_5 in a EuroVector EuroEA Elemental Analyzer. Particulate matter in the Baltic Sea was reported to be free of carbonate (Schneider et al., 2002), and thus, the filters were not acidified prior to analysis. C, N, and S contents were calculated by comparison with peak areas from standards. Standard deviations were ± 2% for C and ± 5% for N and S.

2.3 Lipid analysis

¾ of each filter was extracted (3 x 20 min) with dichloromethane (DCM)/methanol (MeOH) (40 ml; 3:1, v:v) in a CEM Mars 5 microwave (Matthews, NC, USA) at 60°C and 800 W. The freeze dried residue of the cyanobacterial bloom was extracted (3 x 10 min) with DCM/MeOH (10 ml; 3:1, v:v) and ultrasonication. All extracts were combined.

An aliquot of each filter extract and the bloom extract was acetylated using Ac_2O and pyridine (1:1, v:v) for 1 h at 50° C and then overnight at room temperature. The mixture was dried under vacuum and analyzed for BHPs using liquid chromatography-mass spectrometry (LC-MS).

Another aliquot of each filter extract was separated into a hydrocarbon (F1), an alcohol and ketone (F2) and a polar fraction (F3) using column chromatography. The column (Ø ca. 1 cm) was filled with 7.5 g silica gel 60, samples were dried on ca. 500 mg silica gel 60 and placed on the column. The fractions were eluted with 30 ml *n*-hexane/DCM 8:2 (v:v, F1), 30 ml DCM/EtOAC 9:1 (v:v, F2) and 100 ml DCM/MeOH 1:1, (v:v) followed by additional 100 ml MeOH (F3). F2 was dried and derivatized using a BSTFA/pyridine 3:2 (v:v) mixture for 1 h at 40°C. 50% of the polar fraction F3 was further fractionated to obtain PLFA (F3.3) according to Sturt et al. (2004). Briefly, the column was filled with 2 g silica gel 60 and stored at 200°C until use. The F3 aliquot was dried on ca. 500 mg silica gel 60 and placed on the column. After successive elution of the column with 15 ml DCM and 15 ml acetone, the PLFA fraction was eluted with 15 ml MeOH (F3.3). F3.3 was transesterified using trimethylchlorosilane (TMCS) in MeOH (1:9; v:v) for 1 h at 80°C. In the resulting fatty acid methyl ester (FAME) fractions, double bond positions in monounsaturated compounds were determined using dimethyldisulfide (DMDS; Carlson et al., 1989;Gatellier et al., 1993). The samples were dissolved in 200 µl DMDS, 100 µl *n*-hexane, and 30 µl I_2 solution (60 mg I_2 in 1 ml Et_2O) and derivatized at 50°C for 48 h. Subsequently, 1 ml of *n*-hexane and 200 µl of $NaHSO_4$ (5% in water) were added and the *n*-hexane extract was pipetted off. The procedure was repeated 3 x, the *n*-hexane extracts were combined, dried on ca. 500 mg silica gel 60 and put onto a small column (ca. 1 g silica gel 60). For cleaning, the *n*-hexane extract was eluted with ten dead volumes of DCM. F1, F2, F3.3 and the samples treated with DMDS were analyzed using gas chromatography-mass spectrometry (GC-MS).

2.4 Gas chromatography-mass spectrometry (GC-MS) and GC-combustion isotope ratio mass spectrometry (GC-C-IRMS)

GC-MS was performed using a Varian CP-3800 chromatograph equipped with a Phenomenex Zebron ZB-5MS fused silica column (30 m x 0.32 mm; film thickness 0.25 µm) coupled to a

Varian 1200L mass spectrometer. Helium was used as carrier gas. The temperature program started at 80° C (3 min) and ramped to 310° C (held 25 min) with 4° C min^{-1}. Compounds were assigned comparing mass spectra and retention times to published data. Concentrations were determined by comparison with peak areas of squalane (F2 and F3) and *n*-eicosane-D42 (F1) as internal standards.

Compound specific stable carbon isotope ratios of biomarkers in F2 and F3.3 were measured (2x) using a Thermo Trace GC gas chromatograph coupled to a Thermo Delta Plus isotope ratio mass spectrometer. The GC was operated under the same conditions and with the same column as for GC-MS. The combustion reactor contained CuO, Ni and Pt and was operated at 940° C. Isotopic compositions are reported in standard delta notation relative to the Vienna PeeDee Belemnite (V-PDB) and were calculated by comparison with an isotopically known CO_2 reference gas. GC-C-IRMS precision and linearity was checked daily using an external *n*-alkane isotopic standard (provided by A. Schimmelmann, Indiana University).

2.5 Liquid chromatography-mass spectrometry (LC-MS)

LC-MS was performed using a Varian Prostar Dynamax HPLC system fitted with a Merck Lichrocart (Lichrosphere 100; reversed phase (RP) C_{18e} column [250 x 4 mm]) and a Merck Lichrosphere pre-column of the same material coupled to a Varian 1200L triple quadrupole mass spectrometer (both Varian). Used solvents were MeOH/water 9:1 (v:v; solvent A) and MeOH/propan-2-ol 1:1 (v:v; solvent B), and all solvents were Fisher Scientific HPLC grade. The solvent gradient profile was 100% A (0-1 min) to 100% B at 35 min, then isocratic to 60 min. The MS was equipped with an atmospheric pressure chemical ionization (APCI) source operated in positive ion mode (capillary temperature 150° C, vaporizer temperature 400° C, corona discharge current 8 µA, nebulizing gas flow 70 psi, auxiliary gas 17 psi). In SIM (single ion monitoring) mode, ions obtained from acetylated BHP peaks in the samples were compared to authentic BHP standards with known concentration (acetylated BHP and aminotriol) to determine BHP concentrations (external calibration). Amino BHPs had a 7x higher response factor than non-amino BHPs and concentrations in the samples were corrected accordingly. Comparisons with elution times of previously identified compounds further aided in BHP assignment. The quantification error is estimated to be ± 20%.

2.6 Principle Component Analysis (PCA)

PCA was based on the relative abundance of individual components in different water depths and was performed using R (version 3.0.2, 2013-09-25) with the "princomp" module (The R Foundation, 2014).

3 Results

3.1 Physicochemical parameters of the water column

In summer 2011, the Landsort Deep showed a strong vertical stratification (Fig. 2). The oxic zone consisted of the uppermost 80 m and was divided by a strong thermocline into a warm surface layer (~0-10 m) and a cold winter water layer (~10-60 m). The halocline was located between 60 m and 80 m. O_2 concentrations rapidly decreased from >8 ml l^{-1} at ~50 m to <0.2 ml l^{-1} at ~80 m, defining the upper boundary of the suboxic zone (Tyson and Pearson, 1991). H_2S was first detected at 83 m. Because O_2 concentrations could methodologically only be measured in the complete absence of H_2S, oxygen could not be traced below this depth. Therefore, the lower boundary of the suboxic zone was defined to be at 90 m, where H_2S concentrations were sharply increasing. The upper suboxic zone also showed a sharp peak in turbidity that is possibly caused by precipitation of Fe and Mn oxides (Dellwig et al., 2010) or zero-valent sulfur (Kamyshny Jr. et al., 2013) and can be used as an indicator for the O_2-H_2S transition (Kamyshny Jr. et al., 2013). The anoxic zone extends from 90 m to the bottom and is characterized by the complete absence of O_2 and high concentrations of H_2S and CH_4.

CH_4 was highest in the deep anoxic zone, decreased strongly towards the suboxic zone but was still present in minor concentrations in the oxic zone. A small CH_4 peak was detected at the suboxic-anoxic interface (Fig. 2). Particulate organic carbon (POC) was highest at 10 m (380 µg l^{-1}), decreased to a minimum in the cold winter water layer (48 µg l^{-1}) and showed almost constant values of ~70 µg l^{-1} in the suboxic and anoxic zones.

Generally, we follow the zonation of the Landsort Deep water column as given in Jakobs et al. (2014). We regarded the onset of H_2S as the top of the anoxic zone, however, as this is better supported by our biomarker data (see below).

3.2 Lipid analysis

The PCA analysis separated six groups of biomarkers according to their distribution in the water column (Fig. 3, Section 3.2.1-6). Out of these groups, 18 compounds were selected as representative biomarkers specifying inputs from individual prokaryotes and eukaryotes (with phototrophic, chemotrophic and/or heterotrophic metabolisms). These biomarkers and their distributions are discussed in detail in Section 4.

The concentrations of these compounds are shown in Fig. 4, and compound-specific $\delta^{13}C$ values are given in Table 1. Apart from the biomarker families revealed by PCA, two compound classes, *n*-alkanes and *n*-alkenes in the sea surface layer, and individual BHPs obtained from the water column and a cyanobacterial bloom are reported separately (Fig. 5, Section 3.2.7; Fig. 6a, Section 3.2.8, respectively).

3.2.1 Group 1: surface maximum

The first group is defined by a strong maximum in the surface layer and only minor concentrations in greater depths. A subgroup of 14 compounds exclusively occurs at 10 m water depth (Fig. 3). For the other compounds, abundance in greater water depths increases towards the y-axis. 7-methylheptadecane (52), 24-ethylcholest-5-en-3β-ol (β-sitosterol; 48), 20:4ω6 PLFA (34), 20:5ω3 PLFA (33), 16:1ω7c PLFA (11), and cholest-5-en-3β-ol (cholesterol; 44) were taken as representative for group 1. Among these compounds, 16:1ω7 PLFA and cholesterol showed the highest concentrations (1154 ng l^{-1} and 594 ng l^{-1}, respectively), and 7-methylheptadecane the lowest (6 ng l^{-1}, Fig. 4). Apart from their maximum in the surface layer, the fate of these biomarkers in deeper water layers differed. 7-methylheptadecane exclusively occurred in the surface layer, whereas 20:4ω6 was traceable throughout the water column. β-sitosterol occurred in the surface and the bottom layers. Unlike the other compounds, cholesterol and 20:5ω3 PLFA did not show a straight decrease with depth, rather are there minor occurrences right above and at the bottom of the suboxic zone, respectively. $\delta^{13}C$ values of all compounds were between -32 and -26 ‰ (Table 1).

3.2.2 Group 2: surface and lower suboxic zone maxima

Group 2 shows a surface maximum like group 1, but exhibits a stronger emphasis of the lower suboxic zone (Fig. 4). With the exception of 16:7ω7t, all compounds were chosen for further consideration. 4α,23,24-trimethyl-5α-cholest-22E-en-3β-ol (dinosterol; 49) and gammacer-3β-ol (tetrahymanol; 50) had their maximum concentration in the surface water (dinosterol: 66 ng l^{-1}; tetrahymanol: 42 ng l^{-1}) and were not detectable in the layers below, until a sharp second maximum occurred at the bottom of the suboxic zone. Concentrations decreased again below the suboxic zone and remained constantly low in the bottom water. Unlike these compounds, *ai* 15:0 PLFA (5), total bacteriohopanepolyols (BHPs; 84), and the hopanoid hydrocarbon hop-22(29)-ene (diploptene; 54) showed steadily increasing concentrations through the suboxic zone and further increasing concentrations in the anoxic zone. The $\delta^{13}C$ values of all compounds were between -35 and -25 ‰ (Table 1).

3.2.3 Group 3: cold winter water layer maximum

The third group showed compounds that peaked in the cold winter water layer at 65 m water depth (Fig. 3). 17:1ω9 PLFA (19) only occurred at 70 m water depth and *n*-C_{21} (61) from 10 to 70 m with a strong peak at 70 m. The 16:0-18:1 (46; Fig. 4) and 18:0-18:1 (47) wax esters only occurred from 65 to 80 m, with a maximum at 65 m (287 ng l^{-1} and 228 ng l^{-1}, respectively). Out of group 3, the 16:0-18:1 wax ester was included into the discussion. $\delta^{13}C$ values of the wax esters were ~ -28‰ (Table 1).

3.2.4 Group 4: oxic zone maximum

Group 4 consisted exclusively of saturated *n*-alkanes from *n*-C_{21} to *n*-C_{36} as well as 26:0 PLFA (43). 26:0 PLFA only occurred at 80 m, whereas all other compounds were abundant from the surface to the upper suboxic zone at 80 m (data not shown). The homologues *n*-C_{27} (74), *n*-C_{29} (76), and *n*-C_{31} (78) show maxima at the surface (21 - 30 ng l^{-1}). For the other compounds, maxima were either located at 65 or 70 m, with highest concentrations for *n*-C_{25} - *n*-C_{36} (10 - 23 ng l^{-1}). Below 80 m, concentrations dropped to constantly low values. As an example, the depth profile of *n*-C_{25} (71) is shown in Figure 4. $\delta^{13}C$ values for these compounds could not be obtained.

3.2.5 Group 5: suboxic zone maximum

Group 5 contained only two compounds, 16:1ω8c PLFA (10) and the *n*-$C_{26:1}$ alkene (72). *n*-$C_{26:1}$ occurred in very low concentrations at 10m, and peaked at 80 and 95 m (7-8 ng l^{-1}). 16:1ω8c PLFA occurred only at 80 and 90 m water depth, with highest values at 80 m (8 ng l^{-1}; Fig. 4), and was chosen for further discussion. $\delta^{13}C$ values of this compound were ~ -45‰ (Table 1).

3.2.6 Group 6: absent in oxic zone, bottom layer maximum

Group 6 consisted of compounds that only occurred in the suboxic zone and below, and increased in concentration into the anoxic zone. An exception is 5α(H)-cholestan-3β-ol (cholestanol; 45), which was also present in the surface layer. 10-me-16:0 PLFA (16), the irregular C_{25} isoprenoid 2,6,10,15,19-pentamethylicosane (PMI) and three unsaturated derivatives thereof (PMI Δ; 53), 2,3-di-*0*-isopranyl *sn*-glycerol diether (archaeol; 51), and cholestanol were considered for further discussion. For all compounds, maxima were detected in the anoxic zone, with highest concentrations observed for cholestanol (35 ng l^{-1}) followed

by 10-me-16:0 PLFA (10 ng l^{-1}), PMI and PMI Δ (8 ng l^{-1}) and archaeol (1 ng l^{-1}). 10-me-16:0 PLFA shows, compared to other compounds, a slight ^{13}C depletion in the anoxic zone (-35.4 ‰; Table 1). Concentrations of archaeol, PMI, and PMI Δ were too low to determine $\delta^{13}C$.

3.2.7 *n*-alkanes and *n*-alkenes in the sea surface layer

The concentrations of *n*-alkanes and *n*-alkenes in the surface sample (10 m water depth) are given in Fig. 5. The longest *n*-alkane chain was *n*-C_{36}, and odd carbon numbers dominated over even. Highest concentrations were found for *n*-C_{27} (21 ng l^{-1}), *n*-C_{29} (30 ng l^{-1}), and *n*-C_{31} (26 ng l^{-1}). The longest *n*-alkene chain was *n*-$C_{26:1}$, and highest *n*-alkene concentrations were measured for *n*-$C_{23:1}$ (3 ng l^{-1}) and *n*-$C_{25:1}$ (3 ng l^{-1}).

3.2.8 Water-column profiles of BHPs

In the Landsort Deep, seven individual BHPs were identified (Fig. 6a). In all samples, bacteriohopane-32,33,34,35-tetrol (BHT) accounted for the greatest portion of the total BHPs (88- 94%). An as yet uncharacterized BHT isomer, BHT II, was present only below 70 m and showed its highest relative abundance (~2 %) between 70 and 90 m. BHT cyclitol ether, BHT glucosamine, and 35-aminobacteriohopane-32,33,34-triol (aminotriol) were present throughout the water column. BHT cyclitol ether and BHT glucosamine were most abundant in the oxic zone (ca. 1-4%), but showed only minor abundances (< 1%) below. Aminotriol was elevated at 65 and 420 m (~7 and ~5%, respectively). 35-aminobacteriohopane-31,32,33,34-tetrol (aminotetrol) occurred throughout the suboxic and anoxic zones, whereas 35-aminobacteriohopane-30,31,32,33,34-pentol (aminopentol) was observed only at 90 m and below. Both, aminotetrol and aminopentol showed minor relative abundances of ~2% and <1% of the total BHPs, respectively (Jakobs et al. 2014).

For comparison, the major phytoplankton species from a cyanobacterial bloom in the Gotland Deep (2012) were determined by microscopy (HELCOM manual, 2012) and the POM was analysed for BHPs. This reference biomass contained mainly *Aphanizonemon* and, to a smaller extent, *Anabaena* and *Nodularia*, which were accompanied by dinoflagellates. Three BHPs were observed in the bloom POM (Fig. 6b). Among these compounds, the most abundant was BHT (~86 %), followed by BHT cyclitol ether (~10%), and BHT glucosamine (~4%).

4 Discussion

In the following, we discuss several aspects of the biomarker profiles with respect to their significance as tracers for the relevant biota and biogeochemical processes in stratified water columns.

4.1 Water-column redox zones as reflected by cholestanol/cholesterol ratios

Different redox states of the Landsort Deep water column and the associated microbial processes are reflected by the profiles of cholesterol and its diagenetic product, cholestanol (Fig. 4, groups 1 and 6, respectively). Cholesterol is synthesized by various eukaryotic phyto- and zooplankton and higher plants (Parrish et al., 2000) and abundant in water columns and sediments. In sediments as well as in stratified water columns, stanols are produced from sterols by anaerobic bacterial hydrogenation (Gaskell and Eglinton, 1975; Wakeham, 1989) and by the abiotic reduction of double bonds by reduced inorganic species such as H_2S (Hebting et al., 2006; Wakeham et al., 2007). Therefore, cholestanol/cholesterol ratios typically increase under more reducing conditions. In the Black Sea, low ratios of ~0.1 were associated with oxygenated surface waters, the suboxic zone showed ratios between 0.1 and 1, whereas the anoxic zone revealed values >1 (Wakeham et al., 2007). In the Landsort Deep, the cholestanol/cholesterol ratios showed a slight increase with depth from the surface towards the suboxic zone, but always remained <0.1 (Fig. 4). Below, the values increased to ~0.3 in the suboxic zone, and further to a maximum of 0.45 in the anoxic zone. Whereas the ratios in the Landsort Deep are considerably lower than in the Black Sea, the depth trend still clearly mirrors the changes from oxic to suboxic, and further to anoxic conditions. It is also interesting to note that total cholesterol and cholestanol concentrations in the Landsort Deep were ten- and fourfold higher, respectively, as in the Black Sea (Wakeham et al., 2007).

4.2 Phototrophic primary production

As expected, *in situ* biomarkers for phototrophic organisms were most abundant in the surface layer and are pooled in PCA group 1. 20:4ω6 PLFA is a biomarker traditionally assigned to eukaryotic phytoplankton (Nanton and Castell, 1999; Lang et al., 2011) and organisms grazing thereon, such as protozoa (Findlay and Dobbs, 1993; Pinkart et al., 2002; Risse-Buhl et al., 2011). 20:5ω3 PLFA is known to be a major compound in diatoms (Arao and Marada, 1994; Dunstan et al., 1994) and high concentrations of these PLFAs, as observed in the surface layer of the oxic zone, are in good agreement with such an authochthonous plankton-based source.

7-methylheptadecane is a characteristic marker for cyanobacteria (Shiea et al., 1990; Köster et al., 1999). Its most likely source are members of the subclass Nostocophyceae that were often reported to produce isomeric mid-chain branched alkanes, including 7-methylheptadecane (Shiea et al., 1990; Hajdu et al., 2007; Liu et al., 2013). Nostocophyceae are key members of the photoautotrophic community in the Baltic Sea. Particularly the filamentous genera *Nodularia* and *Aphanizonemon* (see 3.2.8), and the picocyanobacterium *Synechococcus* play a major role in blooms during summer time (Stal et al., 2003; Labrenz et al., 2007). The importance of cyanobacteria in the surface layer of the Landsort Deep is further reflected by the presence of $C_{21:1}$, $C_{23:1}$ and $C_{25:1}$ *n*-alkenes (Fig. 5). These compounds have been reported from *Anacystis* (Gelpi et al., 1970) and *Oscillatoria* (Matsumoto et al., 1990). *Oscillatoria vaucher* is also known to occur in the Baltic Sea, but is of only minor abundance (Kononen et al., 1996; Vahtera et al., 2007).

Unlike the *n*-alkenes that only occurred in the surface layer, long-chain *n*-alkanes were present in the whole water column, with high abundances in the oxic zone. Long-chain *n*-alkanes with a strong predominance of the odd-numbered *n*-C_{25} to *n*-C_{36} homologues (Eglinton and Hamilton, 1967; Bi et al., 2005) and β-sitosterol (Volkman, 1986) are typical components of higher plant lipids, thus indicating continental runoff and/or aeolian input of terrigenous OM into the Landsort Deep. *n*-C_{27}, *n*-C_{29}, and *n*-C_{31} showed surface maxima (not shown), indicating similar sources as for β-sitosterol and a contribution of land plant leaf waxes. Other than β-sitosterol, most *n*-alkanes peaked between 65 and 70 m (*n*-C_{25} for example; Fig. 4). Apart from the surface peaks, this is also true for *n*-C_{27}, *n*-C_{29}, and *n*-C_{31} . A possible explanation is the accumulation of terrigenous higher plant particles accumulating at the pycnocline, where density differences were highest (MacIntyre et al., 1995)

4.3 Phototrophic vs. heterotrophic dinoflagellates, and ciliates

The distribution of dinoflagellates and, most likely, ciliates in the water column is reflected by two specific biomarkers, dinosterol and tetrahymanol (see 3.2.2, Fig. 4). Dinosterol is mainly produced by dinoflagellates (Boon et al., 1979), although it was also reported in minor abundance from a diatom (*Navicula* sp., Volkman et al., 1993). The dinosterol concentrations in the Landsort Deep showed a bimodal distribution. The strong peak in the surface layer of the oxic zone probably represents contributions from phototrophic dinoflagellates. Plausible candidates are *Peridiniella catenata* and *Scrippsiella hangoei*, both of which are involved in the spring phytoplankton blooms in the central Baltic Sea (Wasmund et al., 1998; Höglander et al., 2004). The latter species was previously reported to produce dinosterol (Leblond et al.,

2007). However, *P. catenata* as well as *S. hangoei* are virtually absent below 50 m water depth (Höglander et al., 2004) and can thus not account for the second peak of dinosterol at the suboxic-anoxic transition zone. An accumulation of surface-derived dinosterol at the bottom of the suboxic zone is unlikely, as the pycnocline and thus, the strongest density discontinuity, is located at 60-70 m water depth, i.e. about 20 m above. Dinosterol is absent in the pycnocline and only occurs from the bottom of the suboxic zone on and below. Instead, a likely source of dinosterol at this water depth are heterotrophic dinoflagellates that are abundant in the suboxic zones of the central Baltic Sea (Anderson et al., 2012). Due to their enhanced productivity, these environments provide good conditions to sustain communities of eukaryotic grazers (Detmer et al., 1993). A possible candidate, *Gymnodinium beii*, was described from the suboxic zones of the central Baltic Sea (Stock et al., 2009). Indeed, several *Gymnodinium* species are known to be heterotrophs (Strom and Morello, 1998) and some have been reported to produce dinosterol (Mansour et al., 1999). Like cholesterol and β-sitosterol, dinosterol was also found in the anoxic zone at 400 m water depth. The production of these compounds at this depth is unlikely, as the synthesis of sterols requires oxygen (Summons et al., 2006). Hence, the observed sterol occurrences probably reflect transport through the water column.

A similar concentration distribution as for dinosterol was observed for tetrahymanol. Tetrahymanol is known to be produced by ferns, fungi, and bacteria such as the purple non-sulfur bacterium *Rhodopseudomonas palustris* (Zander et al., 1969; Kemp et al., 1984; Kleemann et al., 1990; Sinninghe Damsté et al., 1995; Eickhoff et al., 2013). Moreover, ciliates ubiquitously produce tetrahymanol as a substitute for cholesterol when grazing on prokaryotes instead of eukaryotes such as algae (Conner et al., 1968; Boschker and Middelburg, 2002). This is also a feasible scenario for the Baltic Sea where the ciliate genera *Metopus*, *Strombidium*, *Metacystis*, *Mesodinium*, and *Coleps* are abundant in the suboxic zone and at the suboxic-anoxic interface (Detmer et al., 1993; Anderson et al., 2012). Unidentified ciliates also occurred in the anoxic waters of the Landsort Deep (Anderson et al., 2012). Members of the genus *Rhodopseudomonas*, a possible alternative source of tetrahymanol, have so far not been identified in the suboxic zone (Labrenz et al., 2007; Thureborn et al., 2013). We therefore regard bacterivorous ciliates living under suboxic to anoxic conditions as the most likely source of tetrahymanol in the suboxic zone and below. Likewise, ciliates feeding on chemoautotrophic bacteria were assumed as producers of tetrahymanol in the suboxic zone of the Black Sea (Wakeham et al., 2007). The situation is somewhat different in the surface waters, where tetrahymanol shows its maximum concentrations at 10 m water

depth. Although *Rhodopseudomonas* and other purple non-sulfur bacteria usually occur under oxygen deficient conditions, they have been genetically identified in the surface water of the Landsort Deep (Farnelid et al., 2009) and thus have to be considered as potential producers of tetrahymanol. Furthermore, cholesterol is abundant in the surface waters and could be incorporated by ciliates instead of tetrahymanol. However, some ciliates seem to prefer prokaryotes as a prey. Sinking agglomerates of cyano- and other bacteria are known to be covered by feeding ciliates (Gast and Gocke, 1988). Hence, in addition to *R. palustris*, ciliates grazing selectively on cyanobacteria would plausibly explain the abundance of tetrahymanol in the shallow waters of the Landsort Deep.

$\delta^{13}C$ values of tetrahymanol revealed an opposite trend as compared to dinosterol. While dinosterol became isotopically more negative with depth (-29.9 to -32.0‰), tetrahymanol became more positive (-28.7 to -25.9‰) and showed its highest $\delta^{13}C$ values in the anoxic zone. Although ciliates and dinoflagellates are both grazers at the suboxic-anoxic interface, they seem to occupy different ecological niches and feed on different bacterial sources.

4.4 Heterotrophs in the cold winter water layer

The only biomarkers with enhanced concentrations in the deep cold winter water layer are wax esters (e.g. 16:0-18:1 wax ester, Fig. 4), and, to a minor extent, cholesterol and 20:5ω3 PLFA. As the pycnocline, and thus a strong density discontinuity, is also located at this depth, an accumulation of settling organic debris containing these compounds has to be considered (MacIntyre et al., 1995). Living organisms, however, may be also be plausible sources. Known producers of wax esters and cholesterol are copepods (Lee et al., 1971; Sargent et al., 1977; Kattner and Krause, 1989; Nanton and Castell, 1999; Falk-Petersen et al., 2002) which are often abundant at density layers where they feed on accumulated aggregates (MacIntyre et al., 1995). These organisms synthesize wax esters with total chain lengths between 28 and 44 carbon atoms (Lee et al., 1971; Kattner and Krause, 1989; Falk-Petersen et al., 2002), several of which were present in the Landsort Deep (data not shown in Fig. 4), with roughly the same distribution as the most prominent 16:0-18:1. Although copepods migrate through the water column, particularly those rich in wax esters prefer deep water or near-surface cold water (Sargent et al., 1977), which is in full agreement with the high amounts of these compounds in the cold winter water layer. Copepods are abundant and diverse in the Baltic Sea, with major species being *Pseudocalanus elongatus*, *Temora longicornis*, and *Acartia* spp. (Möllmann et al., 2000; Möllmann and Köster, 2002). Like the wax esters, the 20:5ω3 PLFA shows higher concentrations in the cold winter water layer, but it is also abundant in the surface and at the

suboxic-anoxic interface (Fig. 4). Copepods are also known to feed on diatoms and incorporate their specific fatty acids such as 20:5ω3 PLFA largely unchanged into their own tissues (Kattner and Krause, 1989). Dinoflagellates are also known producers of 20:5ω3 PLFA (Parrish et al., 1994; Volkman et al., 1998) and may be an alternative source in the surface layer and at the suboxic-anoxic interface, which is supported by a good correlation with dinosterol at these depths.

Unlike the abovementioned compounds, all other selected biomarkers show particularly low concentrations in the cold winter water layer. This is also true for widespread compounds such as the 16:1ω7c PLFA which is produced by eukaryotes (Pugh, 1971; Shamsudin, 1992) as well as prokaryotes (Parkes and Taylor, 1983; Vestal and White, 1989). While a mixed origin of 16:1ω7c PLFA has to be assumed for the oxic zone, a bacterial source is more probable in the suboxic zone and in the anoxic zone. Regardless of the biological source, a very low amount of this ubiquitous FA (Fig. 4) indicates that the cold winter water layer of the Landsort Deep does not support abundant planktonic life. Based on microscopy, similar observations have been made for the cold winter water layers of the Gotland, Bornholm and Danzig Basins (Gast and Gocke, 1988, and citations therein).

4.5 BHPs as indicators for aerobic and anaerobic metabolisms

Bacteria are the only known source of BHPs (Kannenberg and Poralla, 1999). Although the biosynthesis of BHPs and their precursor, diploptene (both plotting in group 2), does not require oxygen, the production of hopanoids was long assumed to be restricted to aerobic bacteria, as reports from facultatively or strictly anaerobic bacteria were initially missing. More recently, however, planctomycetes (Sinninghe Damsté et al., 2004), metal reducing *Geobacter* (Fischer et al., 2005), and sulfate reducing *Desulfovibrio* (Blumenberg et al., 2006; 2009; 2012) were identified as anaerobic producers of BHPs. In the Landsort Deep, cyanobacteria are abundant in the surface water layer and may be considered as a major source of BHPs (cf. Talbot et al., 2008; Welander et al., 2010). Evidence for such cyanobacterial BHP contributions may come from our analysis of a Gotland Deep bloom from summer 2012 (see 3.2.7). BHPs identified in this bloom were BHT, BHT cyclitol ether, and BHT glucosamine (Fig. 6b) which is in line with the BHP composition of the Landsort Deep surface layer (Fig. 6a). These three cyanobacterial BHPs were present throughout the Landsort Deep water column, although they were minor in the suboxic zone and below. In addition, the surface layer contained aminotriol that was also present in the whole water column. Aminotriol is an abundant BHP produced by various bacteria (e.g. Talbot and

Farrimond, 2007, and references therein), indicating that organisms other than cyanobacteria may contribute BHP to the surface layer.

A further notable feature is the occurrence of BHT II at 70 m and below. The source of BHT II is not fully resolved yet. It was recently related to planctomycetes, especially those performing anaerobic ammonium oxidation (anammox) in sediments (Rush et al., 2014). Anammox bacteria can also be traced by 10-me16:0 PLFA and ladderane PLFAs (not studied here; Sinninghe Damsté et al., 2005; Schubert et al., 2006). 10-me16:0 PLFA shows indeed a peak at the lower suboxic zone, where BHT II is abundant. However, 10-me16:0 PLFA may also be contributed by sulfate reducing bacteria (see 4.6) and no evidence for anammox has been observed in the water column of the Landsort Deep from molecular biological studies so far (Hietanen et al., 2012; Thureborn et al., 2013). Regardless of the biological source , BHT II was described from stratified water columns of the Arabian Sea, Peru Margin and Cariaco Basin (Sáenz et al., 2011) and the Gotland Deep (Berndmeyer et al., 2013) and has therefore been proposed as a proxy for stratified water columns. This hypothesis has been adopted to reconstruct the development of water-column stratification in the Baltic Sea during the Holocene (Blumenberg et al., 2013).

Like BHT II, aminotetrol and aminopentol are absent from the surface layer (Fig. 6 a). Whereas both BHPs are biomarkers for methanotrophic bacteria, the latter typically occurs in type I methanotrophs (Talbot et al., 2001). The presence of type I methanotrophic bacteria is further supported by the co-occurrence of the specific 16:1ω8c PLFA (Nichols et al., 1985; Bowman et al., 1991; Bowman et al., 1993) and its considerably depleted $\delta^{13}C$ value (-45.4‰).

Whereas a major *in situ* production of BHPs in the suboxic zone is evident from our data, the sources of BHPs in the anoxic zone are more difficult to establish. BHPs in the anoxic zone may partly derive from sinking POM as well as being newly produced by anaerobic bacteria. The further may apply for BHT cyclitol ether and BHT glucosamine which seem to derive from cyanobacteria thriving in the oxic zone, as discussed above. Aminotriol, aminotetrol, and aminopentol, however, are known products of sulfate reducing bacteria (Blumenberg et al., 2006; 2009; 2012) and may have their origin within the anoxic zone. This interpretation is supported by the close correlation of the total BHPs with the *ai*-15:0 PLFA, which is considered as indicative for sulfate reducers (see 4.6; both compounds plotted in the same PCA group 2). Thus, the anoxic zone of the Landsort Deep is likely an active source for BHPs rather than solely being a pool for transiting compounds.

4.6 Microbial processes in the anoxic zone

Sulfate reducing bacteria were traced using *ai*-15:0 PLFA and 10-me-16:0 PLFA (Parkes and Taylor, 1983; Taylor and Parkes, 1983; Vainshtein et al., 1992). The high abundance of *ai*-15:0 PLFA in the surface layer (Fig. 4) is surprising at first glance, as sulfate reducers are not supposed to thrive in oxic environments. However, these bacteria were previously reported from oxygenated surface waters of the Gotland Deep where they were associated with sinking cyanobacterial agglomerates (Gast and Gocke, 1988). 10-Me-16:0 PLFA, otherwise, is absent from the oxic zone (Fig. 4). This FA was reported to occur in *Desulfobacter* and *Desulfobacula* (Taylor and Parkes, 1983; Kuever et al., 2001), both strictly anaerobic organisms (Szewzyk and Pfennig, 1987; Widdel, 1987; Kuever et al., 2001). Indeed, *Desulfobacula toluolica* was genetically identified by Labrenz et al. (2007) in suboxic and anoxic waters of the central Baltic Sea.

In addition to the bacterial FA, two archaeal *in situ* biomarkers, archaeol and PMI, were identified. Archaeol is the most common ether lipid in archaea, but is especially abundant in euryarchaeotes, including methanogens (Tornabene and Langworthy, 1979; Koga et al., 1993). Likewise, PMI and its unsaturated derivatives are diagnostic for methanogenic euryarchaeotes (Tornabene et al., 1979; De Rosa and Gambacorta, 1988; Schouten et al., 1997). In the Landsort Deep, both compounds are virtually absent in the oxic zone, and increase in abundance with depth through the suboxic zone (Fig. 3). The same trend has been described for PMI in the Black Sea (Wakeham et al., 2007) and the presence of euryarchaeota in Landsort Deep anoxic waters has recently been proven by Thureborn et al. (2013).

Given the available sample resolution, it is impossible to further elucidate the exact distribution of archaea in the anoxic zone of the Landsort Deep. Likewise, $\delta^{13}C$ values could not be obtained for archaeol and PMI due to low compound concentrations, which excludes statements on inputs of these lipids from archaea involved in the sulfate-dependent anaerobic oxidation of methane (AOM; cf. Hinrichs et al., 1999; Thiel et al., 2001). Whereas it has been shown that AOM is theoretically possible in the anoxic zone of the Landsort Deep and anaerobic methane consumption has recently been demonstrated to occur (Jakobs et al., 2013), a clear evidence for abundant AOM is as yet lacking and requires further investigations focused at the anoxic water bodies of the Baltic Sea.

5 Conclusions

The Landsort Deep in the western central Baltic Sea is characterized by a stratified water column. Marine microbial organisms have adapted to the vertical chemical limitations of their

ecosystems and their distributions in the water column can be reconstructed using diverse *in situ* biomarkers. According to their behavior in the water column, PCA analysis revealed six groups of biomarkers for distinct groups of (micro)organisms and the related biogeochemical processes. Within the oxic zone, a clear preference for the surface layer became obvious for distinctive biomarkers. Among these compounds, 7-methylheptadecane, different alkenes, BHT cyclitol ether, and BHT glucosamine were indicative for the presence of bacterial primary producers, namely cyanobacteria. Dinosterol concentrations and -δ^{13}C values revealed a phototrophic dinoflagellate population in the surface waters, and a second, heterotrophic community thriving at the suboxic-anoxic interface. Similarly, abundant tetrahymanol at the surface indicated ciliates feeding on cyanobacterial agglomerates, but a second maximum at the suboxic-anoxic interface suggested a further ciliate population that grazed on chemo-autotrophic bacteria. The cold winter water layer at the bottom of the oxic zone showed only low concentrations of biomarkers and seemed to be avoided by most organisms, except copepods. In contrast, biomarkers obtained from the suboxic zone reflected a high abundance and diversity of eukaryotes and prokaryotes. Whereas 16:1ω8 PLFA and aminopentol revealed the presence of type I aerobic methane oxidizing bacteria, *ai*-15:0 PLFA, 10-me-16:0 and total BHPs indicated the distribution of sulfate reducing bacteria in the Landsort Deep water column. The close coupling of *ai*-15:0 PLFA with total BHPs suggests that these bacteria represent a major *in situ* source for hopanoids in the anoxic zone. The anoxic zone was further inhabited by most likely euryarchaeota, as shown by the presence of archaeol and PMI and its derivatives. Our study in the water column of the Landsort Deep gives insights into the recent distributions and actual sources of organic matter as reflected by lipid biomarkers. The results may also aid in the interpretation of organic matter preserved in the sedimentary record, and thus help to better constrain changes in the geological history of the Baltic Sea.

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Tables

Compound	$\delta^{13}C$ [‰] oxic zone	suboxic zone	anoxic zone
Group 1			
7-me-17:0 alkane	n.d.	n.d.	n.d.
β-sitosterol	-29.9	n.d.	-30.1
20:4ω6 PLFA	-30.1	-31.7	-31.6
20:5ω3 PLFA	-29.2	n.d.	n.d.
16:1ω7c PLFA	-30.6	-28.0	-28.3
cholesterol	-26.8	-28.9	-31.7
Group 2			
dinosterol	-29.9	-30.9	-32.0
tetrahymanol	-28.7	-27.9	-25.9
ai-15:0 PLFA	-29.3	-32.5	-34.2
diploptene	n.d.	n.d.	n.d.
Group 3			
16:0-18:1 wax ester	-28.1	-28.2	n.d.
Group 5			
16:1ω8 PLFA	n.d.	-45.4	n.d.
Group 6			
cholestanol	-27.8	-28.9	-30.1
10-me-16:0 PLFA	n.d.	-32.5	-35.4
PMI +PMI Δ	n.d.	n.d.	n.d.
archaeol	n.d.	n.d.	-

Tab. 1: $\delta^{13}C$ values of the compounds chosen from the PCA groups. No $\delta^{13}C$ values were available for group 4. N.d. = not detectable.

Figure captions

Fig. 1: Map showing the sampling locations in the central Baltic Sea.

Fig. 2: Physico-chemical characteristics of the Landsort Deep water column in summer 2011. The suboxic zone is shaded light grey. Temperature and methane data were partially taken from Jakobs et al. (2014).

Fig.3: PCA of the relative abundances of compounds in different water depths. Group 1: surface maximum, a subgroup of compounds exclusively occurring at the surface are listed in the box; Group 2: surface and lower suboxic zone maxima; Group 3: cold winter water layer maximum; Group 4: oxic zone high concentrations; Group 5: suboxic zone maximum; Group 6: absent in oxic zone, bottom layer maximum. Compounds chosen for further discussion are marked bold.

Compounds:

1	13:0 PLFA	22	18:4 PLFA	43	26:0 PLFA	64	*n*-$C_{22:1}$
2	*i* 14:0 PLFA	23	18:2 PLFA	**44**	**cholesterol**	65	*n*-$C_{22:0}$
3	14:0 PLFA	24	18:3 PLFA	**45**	**cholestanol**	66	*n*-$C_{23:1}$
4	*i* 15:0 PLFA	25	18:1ω9c PLFA	**46**	**16:0-18.1 wax ester**	67	*n*-$C_{23:0}$
5	***ai* 15:0 PLFA**	26	18:1ω7c PLFA	47	18:0-18:1 wax ester	68	*n*-$C_{24:1}$
6	15:0 PLFA	27	18:1ω6c PLFA	**48**	**β-Sitosterol**	69	*n*-$C_{24:0}$
7	16:4 PLFA	28	18:1ω5c PLFA	**49**	**dinosterol**	70	*n*-$C_{25:1}$
8	*i* 16:0 PLFA	29	18:0 PLFA	**50**	**tetrahymanol**	**71**	***n*-$C_{25:0}$**
9	16:1ω9c PLFA	30	10-me-18:0 PLFA	**51**	**archaeol**	72	*n*-$C_{26:1}$
10	**16:1ω8c PLFA**	31	*i* C19:0 PLFA	**52**	**7-methylheptadecane**	73	*n*-$C_{26:0}$
11	**16:1ω7c PLFA**	32	19:0 PLFA	**53**	**PMI + PMI D**	74	*n*-$C_{27:0}$
12	16:1ω7t PLFA	**33**	**20:5ω3 PLFA**	**54**	**diploptene**	75	*n*-$C_{28:0}$
13	16:1ω5c PLFA	**34**	**20:4ω6 PLFA**	55	*n*-$C_{17:1}$	76	*n*-$C_{29:0}$
14	16:1ω5t PLFA	35	20:3 PLFA	56	*n*-$C_{17:0}$	77	*n*-$C_{30:0}$
15	16:0 PLFA	36	20:3 PLFA	57	*n*-$C_{18:0}$	78	*n*-$C_{31:0}$
16	**10-me-16:0 PLFA**	37	20:1 PLFA	58	*n*-$C_{19:1}$	79	*n*-$C_{32:0}$
17	*i* C17:0 PLFA	38	20:0 PLFA	59	*n*-$C_{19:0}$	80	*n*-$C_{33:0}$
18	*ai* C17:0 PLFA	39	22:6 PLFA	60	*n*-$C_{20:1}$	81	*n*-$C_{34:0}$
19	17:1 PLFA	40	22:4 PLFA	61	*n*-$C_{20:0}$	82	*n*-$C_{35:0}$

20	17:0 PLFA	41	22:0 PLFA	62	*n*-$C_{21:1}$	83	*n*-$C_{36:0}$
21	18:4 PLFA	42	24:0 PLFA	63	*n*-$C_{21:0}$	**84**	**total BHPs**

Fig. 4: Vertical distribution of biomarkers in the Landsort Deep water column. The suboxic zone is shaded grey.

Fig. 5: Concentrations of *n*-alkanes and *n*-alkenes in the Landsort Deep surface layer (oxic zone, 10 m water depth).

Fig. 6: Relative abundances of individual BHPs (as percent of the total) of a) the Landsort Deep water column and b) the Gotland Deep cyanobacterial bloom. Note that [%]-axes start at 85 %. * = data taken from Jakobs et al. (2014).