

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

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# 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 different cyanobacteria and eukaryotes such as 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 players and the related biogeochemical processes in stratified water columns of marginal seas.

## 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

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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, and thus, even the strong inflow from 1993 had only minor effects on Landsort Deep, where stagnating conditions prevailed throughout (Bergström and Matthäus, 1996). The relatively stable stratification in the Landsort Deep provides environments for microbial life within the oxic, suboxic and anoxic zones.

Little is known, however, about the particulate organic matter (POM) sources and biomarker distributions in the Landsort Deep water column (and the Baltic Sea in general), as most studies focus on pollution related markers in particular organisms and sediments (e.g. Beliaeff and Burgeot, 2001; Lehtonen et al., 2006; Hanson et al., 2009). Recent work has given insight into the distributions of bacteriohopanepolyols (BHPs) and phospholipid fatty acids (PLFA) in the water column of the Gotland Deep (eastern central Baltic Sea), but these studies were focused on bacterial methanotrophy (Schmale et al., 2012; Berndmeyer et al., 2013; Jakobs et al., 2014). In these investigations, as well as in our current study, in situ pumping was used for sampling. In situ pumping allows sampling of biomarkers in exactly the water depth where they are produced, thus providing information about the coupling of water column chemistry and microbial life. Several focused in situ biomarker water column studies exist (Schouten et al., 2001; Schubert et al., 2006; Blumenberg et al., 2007; Sáenz et al., 2011; Xie et al., 2014, and others). Comprehensive in situ biomarker reports exist from the Black Sea water column (Wakeham et al., 2007, 2012). These studies gave a wide-ranging overview of various biomarkers and their producers, and identified a close coupling of microorganisms to water layers. With respect to bacterial methane oxidation, the importance of the Baltic Sea suboxic zone for microbial processes was recently confirmed by Jakobs et al. (2013, 2014). The authors also stated the theoretical possibility of sulfate-dependent methane oxidation in the anoxic zone, a process that still has to be proven in 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

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a sampling site for this biomarker study. Here we report the depth profiles of individual lipids from Landsort Deep, providing further insight into the distribution of relevant biota and the connected biogeochemical processes in stratified water columns.

## 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 30 cm; 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 Vn<sub>2</sub>O<sub>5</sub> 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

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peak areas from standards. Standard deviations were  $\pm 2\%$  for C and  $\pm 5\%$  for N and S.

## 2.3 Lipid analysis

$\frac{3}{4}$  of each filter was extracted (3 min  $\times$  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. All extracts were combined.

The freeze dried residue of the cyanobacterial bloom was extracted (3 min  $\times$  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<sub>2</sub>O 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 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 ( $\emptyset$  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; Gatelier et al., 1993). The samples were dissolved in 200  $\mu$ L DMDS, 100  $\mu$ L *n*-hexane, and 30  $\mu$ L I<sub>2</sub> solution (60 mg I<sub>2</sub> in 1 mL Et<sub>2</sub>O) and derivatized at 50 °C for 48 h. Subsequently,

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1 mL of *n*-hexane and 200  $\mu$ L of NaHSO<sub>4</sub> (5% in water) were added and the *n*-hexane extract was pipetted off. The procedure was repeated 3 $\times$ , 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 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  $\times$  0.32 mm; film thickness 0.25  $\mu$ m) coupled to a Varian 1200L mass spectrometer. Helium was used as carrier gas. The temperature program started at 80  $^{\circ}$ C (3 min) and ramped to 310  $^{\circ}$ C (held 25 min) with 4  $^{\circ}$ C min<sup>-1</sup>. Compounds were assigned comparing mass spectra and retention times to published data. Concentrations were determined by comparison with peak areas of internal standards.

Compound specific stable carbon isotope ratios of biomarkers in F2 and F3.3 were measured (2 $\times$ ) 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  $^{\circ}$ 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<sub>2</sub> 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<sub>18e</sub> column [250 mm  $\times$

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4 mm]) and a Merck Lichrosphere pre-column of the same material coupled to a Varian 1200 L triple quadrupole mass spectrometer. 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  $\mu$ A, 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 7 $\times$  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  $\pm 20\%$ .

## 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 ( $\sim 0$ –10 m) and a cold winter water layer ( $\sim 10$ –70 m). The halocline was located between 60 m and 80 m. O<sub>2</sub> concentrations rapidly decreased from  $> 8 \text{ mL L}^{-1}$  at  $\sim 50$  m to  $< 0.2 \text{ mL L}^{-1}$  at  $\sim 80$  m, defining the upper boundary of the suboxic zone (Tyson and Pearson, 1991). H<sub>2</sub>S was first detected at 83 m. Because O<sub>2</sub> concentrations could methodically only be measured in the complete absence of H<sub>2</sub>S, 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<sub>2</sub>S concentrations were sharply increasing. The suboxic zone also showed a sharp peak in turbidity that is possibly

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centrations ( $594 \text{ ng L}^{-1}$ ), and 7-methylheptadecane the lowest ( $6 \text{ ng L}^{-1}$ , Fig. 3). Apart from their maxima in the surface layer, the trend of these biomarkers differed somewhat in deeper water layers. 20 : 4 $\omega$ 6 PLFA was traceable throughout the water column, whereas 7-methylheptadecane exclusively occurred in the surface layer.  $\beta$ -sitosterol occurred in the surface and the bottom layer. Unlike the other compounds, cholesterol did not show a straight decrease with depth, rather are there minor peaks right above and at the bottom of the suboxic zone, respectively. These variations were small, however, and were not considered for grouping the compounds.  $\delta^{13}\text{C}$  values of all compounds were between  $-32$  and  $-26\text{‰}$  (Table 1).

### 3.2.2 Group 2: surface and lower suboxic zone maxima

This group contains only two compounds, 4 $\alpha$ ,23,24-trimethyl-5 $\alpha$ -cholest-22E-en-3 $\beta$ -ol (dinosterol) and gammacer-3 $\beta$ -ol (tetrahymanol). Both had their maximum concentration in the surface water (dinosterol:  $66 \text{ ng L}^{-1}$ ; tetrahymanol:  $42 \text{ ng L}^{-1}$ ) 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. Tetrahymanol was isotopically heavier in the oxic than in the anoxic zone ( $\delta^{13}\text{C}$ :  $-28.1$  vs.  $-25.9\text{‰}$ ), whereas the opposite was observed for dinosterol ( $\delta^{13}\text{C}$ :  $-29.9$  vs.  $-32.0\text{‰}$ ).

### 3.2.3 Group 3: surface maximum, continuous increase in suboxic zone

The third group contains 16 : 1 $\omega$ 7 PLFA, total BHPs, the hopanoid hydrocarbon hop-22(29)-ene (diploptene), and *ai*-15 : 0 PLFA. All these compounds were abundant in the surface layer, with 16 : 1 $\omega$ 7 PLFA showing the highest concentrations ( $1154 \text{ ng L}^{-1}$ ) and diploptene the lowest ( $12 \text{ ng L}^{-1}$ ). A further feature is the continuous increase that extends throughout the suboxic zone and the anoxic zone. *ai*-15 : 0 PLFA shows a slight isotopic depletion in the anoxic zone ( $-34.2\text{‰}$ ) whereas the other compounds of this group showed consistently higher  $\delta^{13}\text{C}$  values of about  $-28$  to  $-30\text{‰}$ .

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### 3.2.4 Group 4: Absent in the oxic zone, bottom layer maximum

10-me-16:0 PLFA, the irregular  $C_{25}$  isoprenoid 2,6,10,15,19-pentamethylcosane (PMI), three unsaturated derivatives thereof (PMI  $\Delta$ ), and 2,3-di-*o*-isopranyl *sn*-glycerol diether (archaeol) showed profiles defined in group four. These compounds were all absent in the oxic zone and only occurred in the suboxic zone and below. In all cases, maxima were detected in the anoxic zone, with highest amounts observed for 10-me-16:0 PLFA ( $10 \text{ ng L}^{-1}$ ) followed by PMI and PMI  $\Delta$  ( $8 \text{ ng L}^{-1}$ ) and archaeol ( $1 \text{ ng L}^{-1}$ ). 10-me-16:0 PLFA shows a slight  $^{13}\text{C}$  depletion in the anoxic zone ( $-35.4\%$ ). Concentrations of archaeol, PMI, and PMI  $\Delta$  were too low to determine  $\delta^{13}\text{C}$ .

### 3.2.5 Others

$5\alpha$  (H)-cholestan- $3\beta$ -ol (cholestanol), 16:0–18:1 wax ester, 16:1 $\omega$ 8 PLFA, and 20:5 $\omega$ 3 PLFA showed individual profiles not related to any of the groups defined above. Cholestanol shows lowest values within the oxic zone, although concentrations start to increase in the cold winter water layer. Maxima occur at the suboxic–anoxic interface ( $33 \text{ ng L}^{-1}$ ) and in the deep anoxic zone ( $35 \text{ ng L}^{-1}$ ). The wax ester shows maximum concentrations ( $286 \text{ ng L}^{-1}$ ) in the cold winter water layer, and a decrease through the suboxic zone. It was absent in the surface layer and in the anoxic zone. 20:5 $\omega$ 3 PLFA has maximum concentrations in the surface layer ( $15 \text{ ng L}^{-1}$ ), remains at relatively high concentrations in the cold winter water layer ( $6 \text{ ng L}^{-1}$ ) and shows a second peak at the suboxic–anoxic interface. 16:1 $\omega$ 8 PLFA is absent in the oxic and anoxic layers. It only occurs in the suboxic zone with a maximum concentration at its top ( $7 \text{ ng L}^{-1}$ ). Of all compounds measured, it shows the lowest  $\delta^{13}\text{C}$  ( $-45.4\%$ ).

### 3.2.6 *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. 4. The longest *n*-alkane chain was *n*- $C_{33}$ , and odd carbon num-

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bers dominated over even. Highest concentrations were found for  $n\text{-C}_{27}$  (21 ng L<sup>-1</sup>),  $n\text{-C}_{29}$  (30 ng L<sup>-1</sup>), and  $n\text{-C}_{31}$  (26 ng L<sup>-1</sup>). The longest  $n$ -alkene chain was  $n\text{-C}_{26:1}$ , highest  $n$ -alkene concentrations were measured for  $n\text{-C}_{23:1}$  (3 ng L<sup>-1</sup>) and  $n\text{-C}_{25:1}$  (3 ng L<sup>-1</sup>).

### 3.2.7 Individual BHPs

In the Landsort Deep, seven individual BHPs were identified (Fig. 5a). 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).

At the Gotland Deep, a cyanobacterial bloom occurred, which consisted mainly of *Aphanizonemon*, to a smaller degree of *Anabaena* and *Nodularia* and was accompanied by dinoflagellates. The phytoplankton species and biomass were determined by the microscopical method according to the manual of HELCOM (2012). Three BHPs were observed in the bloom POM (Fig. 5b). Among these compounds, the most abundant was BHT (~ 86 %), followed by BHT cyclitol ether (~ 10 %), and BHT glucosamine (~ 4 %).

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## 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. 3). Cholesterol is produced by various eukaryotes such as plankton and higher plants (Parrish et al., 2000) and is 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<sub>2</sub>S (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 (Wakeham et al., 2007). 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. 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 showed a clear preference for the surface layer. Among these compounds, 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 3.2.7), 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<sub>21:1</sub>, C<sub>23:1</sub> and C<sub>25:1</sub> *n*-alkenes (Fig. 4). 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).

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). High concentrations of 20 : 4ω6 PLFA, as observed in the surface layer of the oxic zone, are in good agreement with such an autochthonous plankton-based source.

Long-chain *n*-alkanes with a strong predominance of the odd-numbered *n*-C<sub>25</sub> to *n*-C<sub>33</sub> homologues (Eglinton and Hamilton, 1967; Bi et al., 2005) and β-sitosterol (Volkman, 1986) are typical components of higher plant lipids. The occurrence and distributions of these compounds reflect a significant contribution from terrestrial higher plants and thus, continental runoff and/or aeolian input of terrigenous OM into the Landsort Deep.

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### 4.3 Phototrophic vs. heterotrophic dinoflagellates, and ciliates

The distribution of dinoflagellates and ciliates in the water column is reflected by two specific biomarkers, dinosterol and tetrahymanol (see Sect. 3.2.2, Fig. 3). 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 most likely represents 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; Högländer 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 (Högländer et al., 2004) and can thus not account for the second peak of dinosterol at the suboxic-anoxic transition zone. 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).

A similar concentration distribution as for dinosterol was observed for tetrahymanol. Tetrahymanol is produced by ciliates as a substitute for cholesterol when grazing on prokaryotes instead of eukaryotes such as algae (Conner et al., 1968; Boschker and Middelburg, 2002). High concentrations of tetrahymanol were also described for the suboxic zone of the Black Sea (Wakeham et al., 2007), where ciliates were assumed to feed on chemoautotrophic bacteria. This is also a feasible scenario for the Baltic Sea where the ciliate genera *Metopus*, *Strombidium*, *Metacystis*, *Mesodinium*, and *Coleps*

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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). We therefore assume ciliates living under suboxic to anoxic conditions to be the source of tetrahymanol in the suboxic zone and below.

In the Black Sea, tetrahymanol was absent in the surface waters (Wakeham et al., 2007) whereas the Landsort Deep showed the highest concentration at 10 m depth (Fig. 3). The occurrence of tetrahymanol at this depth appears paradox, as cholesterol is also abundant in the surface waters and thus, the ciliates could incorporate it, e.g. through grazing on eukaryote derived OM. On the other hand, 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). Such a selective diet would plausibly explain the abundance of tetrahymanol in the shallow waters of the Landsort Deep.

$\delta^{13}\text{C}$  values of tetrahymanol revealed an opposite trend as compared to dinosterol. While dinosterol became isotopically lighter with depth ( $-29.9$  to  $-32.0\%$ ), tetrahymanol became heavier ( $-28.7$  to  $-25.9\%$ ) and showed its highest  $\delta^{13}\text{C}$  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 cold winter water layer are wax esters (e.g. 16 : 0–18 : 1 wax ester, Fig. 3) and 20 : 5 $\omega$ 3 PLFA. Known producers of wax esters and 20 : 5 $\omega$ 3 PLFA are copepods (Lee et al., 1971; Sargent et al., 1977; Kattner and Krause, 1989; Nanton and Castell, 1999; Falk-Petersen et al., 2002). 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) of which several were present in the Landsort Deep (data not shown in Fig. 3), follow-

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ing the distribution of the most prominent 16 : 0–18 : 1. Particularly copepods 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 : 5 $\omega$ 3 FA shows high concentrations in the cold winter water layer, but it is also abundant in the surface and at the suboxic–anoxic interface (Fig. 3), suggesting multiple biological origins for this compound. Dinoflagellates are known producers of 20 : 5 $\omega$ 3 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 : 1 $\omega$ 7 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 : 1 $\omega$ 7 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. 3) 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, 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

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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 Sect. 3.2.7). BHPs identified in this bloom were BHT, BHT cyclitol ether, and BHT glucosamine (Fig. 5b) which is in line with the BHP composition of the Landsort Deep surface layer (Fig. 5a). 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 BHP sources other than cyanobacteria in 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 bacteria performing anaerobic ammonium oxidation in sediments (Rush et al., 2014), but two recent studies in the Landsort Deep could not give evidence for anammox in the water column of the Landsort Deep (Hietanen et al., 2012; Thureborn et al., 2013). BHT II was also described from stratified water columns of the Arabian Sea, Peru Margin and Cariaco Basin (Sáenz et al., 2011) and the Baltic Sea Gotland Deep (Berndmeyer et al., 2013) and has therefore been proposed as a proxy for stratified water columns. This theory has positively been adopted to reconstruct the water column stratification in the Baltic Sea Holocene development (Blumenberg et al., 2013).

Like BHT II, aminotetrol and aminopentol are absent from the surface layer. 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 : 1ω8 PLFA (Nichols

et al., 1985; Bowman et al., 1991, 1993) and its considerably depleted  $\delta^{13}\text{C}$  value ( $-45.4\text{‰}$ ).

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 Sect. 4.7.). Thus, the anoxic zone of the Landsort Deep is likely an active source for BHPs instead of 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. 3) 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, on the other hand, is absent from the oxic zone. 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.





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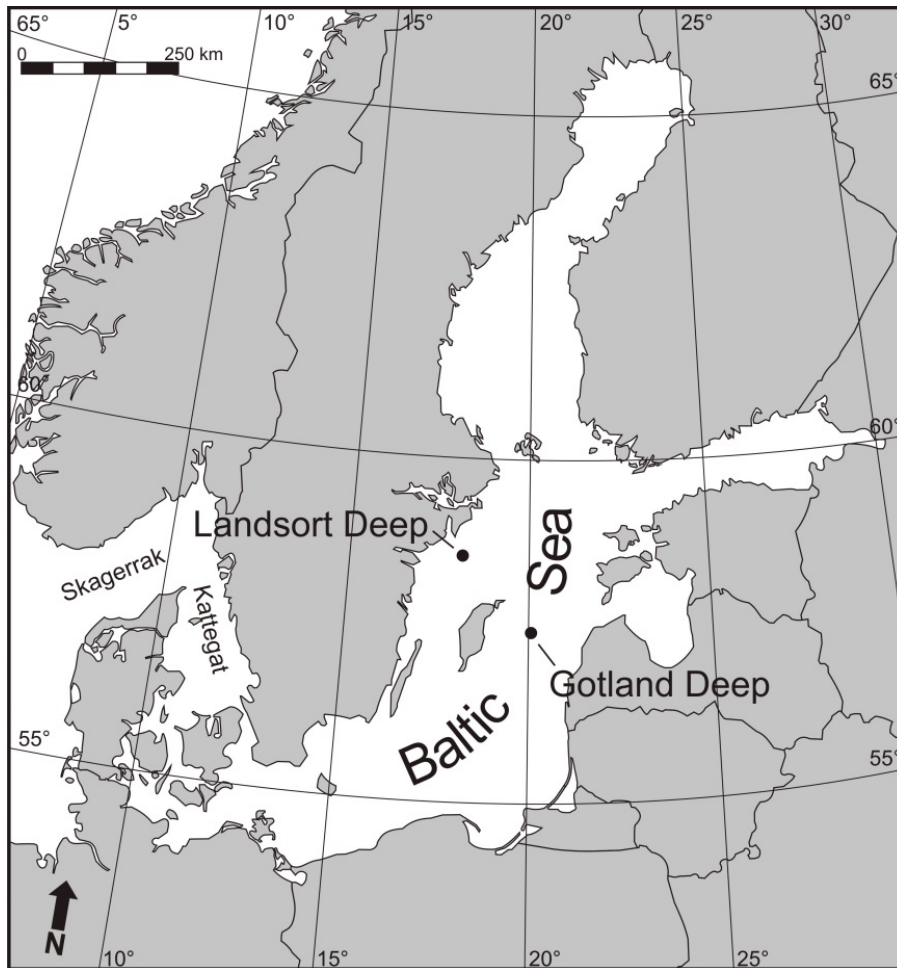
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**Table 1.**  $\delta^{13}\text{C}$  values of the major compounds. The compounds were grouped according to their profile in the water column. N.d. = not detectable.

Compound	$\delta^{13}\text{C}$ [‰]		
	oxic zone	suboxic zone	anoxic zone
<b>Group 1</b>			
cholesterol	−26.8	−28.9	−31.7
7-me-17 : 0 alkane	n.d.	n.d.	n.d.
$\beta$ -sitosterol	−29.9	n.d.	−30.1
20 : 4 $\omega$ 6 PLFA	−30.1	−31.7	−31.6
<b>Group 2</b>			
tetrahymanol	−28.7	−27.9	−25.9
dinosterol	−29.9	−30.9	−32.0
<b>Group 3</b>			
16 : 1 $\omega$ 7 PLFA	−30.6	−28.0	−28.3
diploptene	n.d.	n.d.	n.d.
<i>ai</i> -15 : 0 PLFA	−29.3	−32.5	−34.2
<b>Group 4</b>			
10-me-16 : 0 PLFA	n.d.	−32.5	−35.4
PMI+PMI $\Delta$	n.d.	n.d.	n.d.
archaeol	n.d.	n.d.	n.d.
<b>Others</b>			
cholestanol	−27.8	−28.9	−30.1
20 : 5 $\omega$ 3 PLFA	−29.2	n.d.	n.d.
16 : 0-18 : 1 wax ester	−28.1	−28.2	n.d.
16 : 1 $\omega$ 8 PLFA	n.d.	−45.4	n.d.



**Figure 1.** Map showing the sampling locations in the central Baltic Sea.

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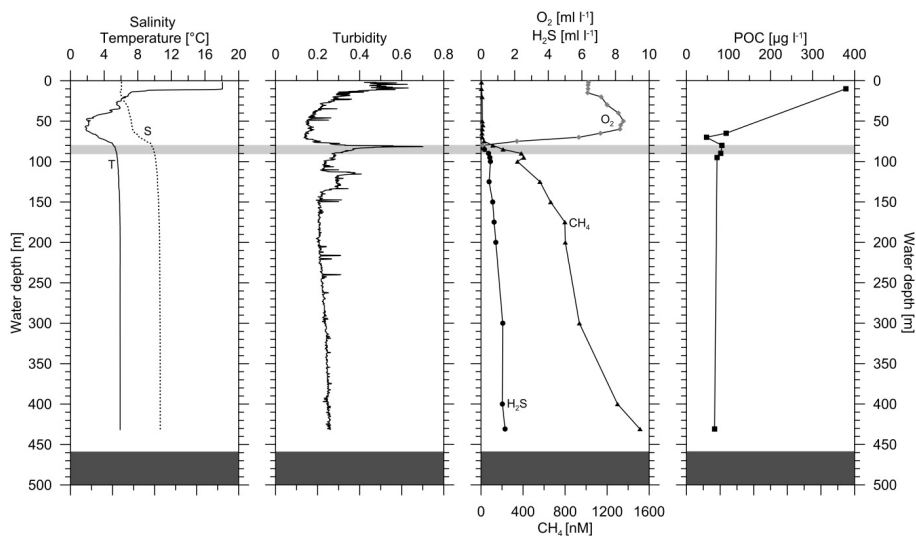
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**Figure 2.** Physico-chemical characteristics of the Landsort Deep water column in summer 2011. The suboxic zone is shaded light grey. Temperature and methane data partially taken from Jakobs et al. (2014).

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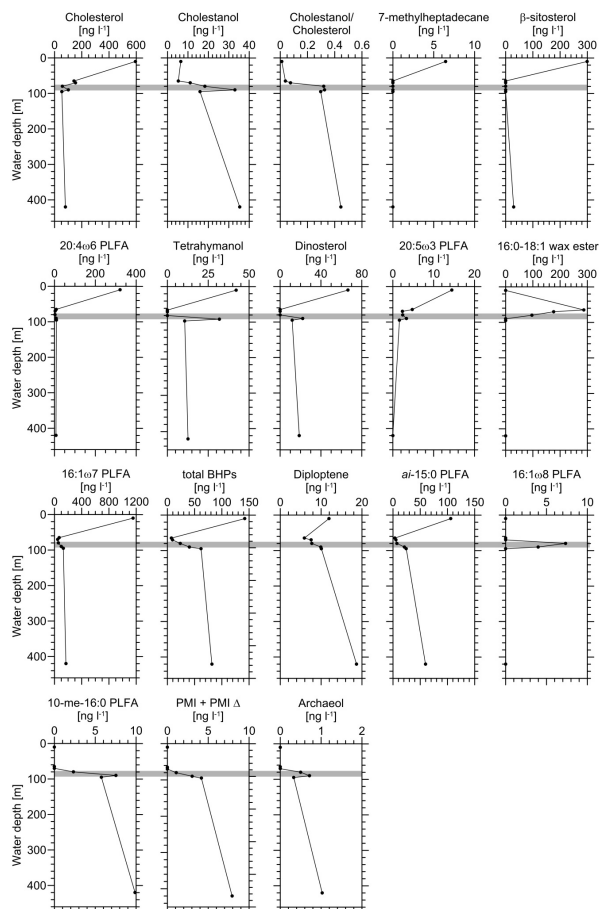
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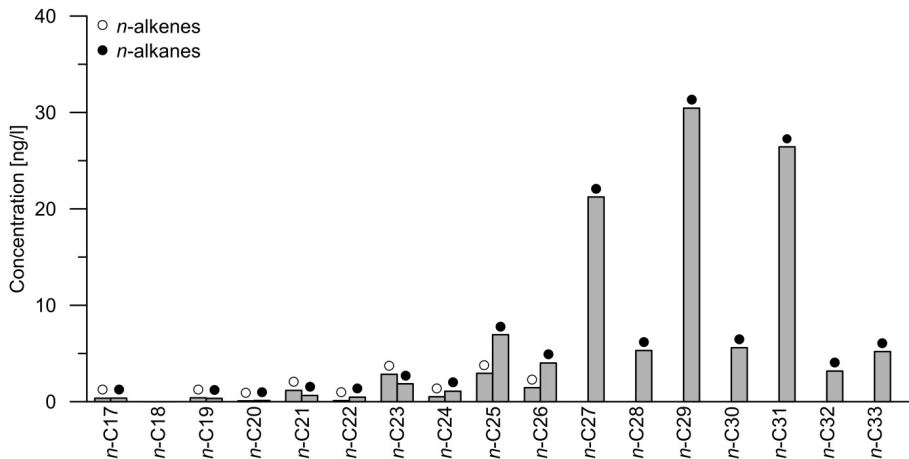
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**Figure 3.** Vertical distribution of biomarkers in the Landsort Deep water column. The suboxic zone is shaded grey.

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**Figure 4.** Concentrations of *n*-alkanes and *n*-alkenes in the Landsort Deep surface layer (oxic zone, 10 m water depth).

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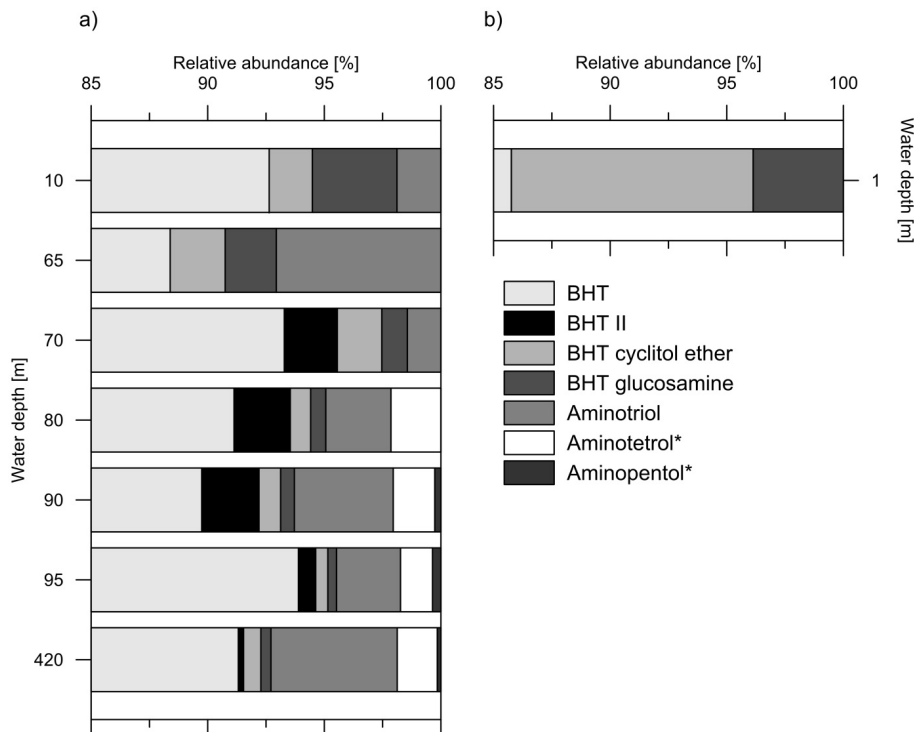
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**Figure 5.** Relative abundances of individual BHPs (as percent of the total) of **(a)** the Landsort Deep water column and **(b)** the Gotland Deep cyanobacterial bloom. Note that [%]-axes start at 85%. \* = data taken from Jakobs et al. (2014).

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