

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

3
4 **C. Berndmeyer¹, V. Thiel¹, O. Schmale², N. Wasmund², and M. Blumenberg^{1,*}**

5
6 [1]{Geobiology Group, Geoscience Center, Georg-August-University Göttingen,
7 Goldschmidtstr. 3, 37077 Göttingen, Germany}

8 [2]Leibniz Institute for Baltic Sea Research Warnemünde (IOW), Seestr. 15, 18199 Rostock-
9 Warnemünde, Germany

10 [*]{present address: Federal Institute for Geosciences and Natural Resources (BGR),
11 Stilleweg 2, 30655 Hannover, Germany}

12 Correspondence to: C. Berndmeyer (christine.berndmeyer@geo.uni-goettingen.de)

13
14
15
16
17
18
19
20
21
22
23
24
25
26
27
28
29
30

1 **Abstract**

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

19
20
21
22
23
24
25
26
27
28
29
30
31
32
33

1 **1 Introduction**

2 The Baltic Sea is a brackish marine marginal Sea with a maximum depth of 459 m in the
3 Landsort Deep (western central Baltic Sea; Matthäus and Schinke, 1999; Reissmann et al.,
4 2009; Fig. 1). A positive freshwater budget and saltwater inflows from the North Sea through
5 Skagerrak and Kattegat lead to a permanent halocline that stratifies the water column of the
6 central Baltic Sea at about 60 m water depth (Reissmann et al., 2009). Major saltwater
7 inflows, as detected in 1993 and 2003, sporadically disturb the stratification in the eastern
8 central Baltic Sea and oxygenate the suboxic zone and deep water. These inflows, however,
9 rarely reach the western central Baltic Sea. Even the strong inflow from 1993 had only minor
10 effects on Landsort Deep, where stagnating conditions prevailed throughout (Bergström and
11 Matthäus, 1996). Therefore, the Landsort Deep offers stable environments for microbial life
12 within the oxic, suboxic and anoxic zones, and provides an excellent study site for the
13 investigation of biomarker inventories that specify stratified water columns.

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

22 For the Baltic Sea water column, biomarker knowledge is limited as most studies so far were
23 focused on pollution related compounds (e.g. Beliaeff and Burgeot, 2001; Lehtonen et al.,
24 2006; Hanson et al., 2009). Recently, we reported the water column distributions and ¹³C-
25 isotopy of individual bacteriohopanepolyols (BHPs) and phospholipid fatty acids (PLFA)
26 from the Gotland Deep, located about 150 km SE of the Landsort Deep in the eastern central
27 Baltic Sea. These studies were aimed at microbial methane turnover and confirmed the
28 importance of the Baltic Sea suboxic zone for bacterial methane oxidation (Schmale et al.,
29 2012; Berndmeyer et al., 2013; Jakobs et al., 2014). The theoretical possibility of sulfate-
30 dependent methane oxidation in the anoxic zone was also stated (Jakobs et al., 2014), but still
31 remains to be proven for the central Baltic Sea water column.

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

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

8

9 **2 Material and methods**

10 **2.1 Samples**

11 Samples were taken during cruise 06EZ/11/05 of R/V *Elisabeth Mann Borghese* in summer
12 2011. The Landsort Deep is located north of Gotland (58°35.0' N 18°14.0' E; Fig. 1). A
13 Seabird sbe911+ CTD system and a turbidity sensor ECO FLNTU (WET Labs) were used for
14 continuous water column profiling. Oxygen and hydrogen sulfide concentrations were
15 measured with Winkler's method and colometrically, respectively (Grasshoff et al., 1983).
16 Filter samples of 65 to 195 L obtained from 10, 65, 70, 80, 90, 95 and 420 m water depth
17 were taken with an *in situ* pump and particulate material was filtered onto precombusted glass
18 microfiber filters (Ø 30cm; 0.7 µm pore size; Munktell & Filtrak GmbH, Germany). Filters
19 were freeze dried and kept frozen at -20° C until analysis.

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

24

25 **2.2 Bulk CNS analysis**

26 Three pieces (Ø 1.2 cm) from different zones of the filters were combusted together with
27 V_{N2}O₅ in a EuroVector EuroEA Elemental Analyzer. Particulate matter in the Baltic Sea was
28 reported to be free of carbonate (Schneider et al., 2002), and thus, the filters were not
29 acidified prior to analysis. C, N, and S contents were calculated by comparison with peak
30 areas from standards. Standard deviations were ± 2% for C and ± 5% for N and S.

31

32 **2.3 Lipid analysis**

1 ¾ of each filter was extracted (3 x 20 min) with dichloromethane (DCM)/methanol (MeOH)
2 (40 ml; 3:1, v:v) in a CEM Mars 5 microwave (Matthews, NC, USA) at 60°C and 800 W. All
3 extracts were combined.

4 The freeze dried residue of the cyanobacterial bloom was extracted (3 x 10 min) with
5 DCM/MeOH (10 ml; 3:1, v:v) and ultrasonication. All extracts were combined.

6 An aliquot of each filter extract and the bloom extract was acetylated using Ac₂O and pyridine
7 (1:1, v:v) for 1 h at 50° C and then overnight at room temperature. The mixture was dried
8 under vacuum and analyzed for BHPs using liquid chromatography-mass spectrometry (LC-
9 MS).

10 Another aliquot of each filter extract was separated into a hydrocarbon (F1), an alcohol and
11 ketone (F2) and a polar fraction (F3) using column chromatography. The column (Ø ca. 1 cm)
12 was filled with 7.5 g silica gel 60, samples were dried on ca. 500 mg silica gel 60 and placed
13 on the column. The fractions were eluted with 30 ml *n*-hexane/DCM 8:2 (v:v, F1), 30 ml
14 DCM/EtOAc 9:1 (v:v, F2) and 100 ml DCM/MeOH 1:1, (v:v) followed by additional 100 ml
15 MeOH (F3). F2 was dried and derivatized using a BSTFA/pyridine 3:2 (v:v) mixture for 1 h
16 at 40°C. 50% of the polar fraction F3 was further fractionated to obtain PLFA (F3.3)
17 according to Sturt et al. (2004). Briefly, the column was filled with 2 g silica gel 60 and stored
18 at 200°C until use. The F3 aliquot was dried on ca. 500 mg silica gel 60 and placed on the
19 column. After successive elution of the column with 15 ml DCM and 15 ml acetone, the
20 PLFA fraction was eluted with 15 ml MeOH (F3.3). F3.3 was transesterified using
21 trimethylchlorosilane (TMCS) in MeOH (1:9; v:v) for 1 h at 80°C. In the resulting fatty acid
22 methyl ester (FAME) fractions, double bond positions in monounsaturated compounds were
23 determined using dimethyldisulfide (DMDS; Carlson et al., 1989; Gatellier et al., 1993). The
24 samples were dissolved in 200 µl DMDS, 100 µl *n*-hexane, and 30 µl I₂ solution (60 mg I₂ in
25 1 ml Et₂O) and derivatized at 50°C for 48 h. Subsequently, 1 ml of *n*-hexane and 200 µl of
26 NaHSO₄ (5% in water) were added and the *n*-hexane extract was pipetted off. The procedure
27 was repeated 3 x, the *n*-hexane extracts were combined, dried on ca. 500 mg silica gel 60 and
28 put onto a small column (ca. 1 g silica gel 60). For cleaning, the *n*-hexane extract was eluted
29 with ten dead volumes of DCM. F1, F2, F3.3 and the samples treated with DMDS were
30 analyzed using gas chromatography-mass spectrometry (GC-MS).

31

32 **2.4 Gas chromatography-mass spectrometry (GC-MS) and GC-combustion** 33 **isotope ratio mass spectrometry (GC-C-IRMS)**

1 GC-MS was performed using a Varian CP-3800 chromatograph equipped with a Phenomenex
2 Zebron ZB-5MS fused silica column (30 m x 0.32 mm; film thickness 0.25 μm) coupled to a
3 Varian 1200L mass spectrometer. Helium was used as carrier gas. The temperature program
4 started at 80° C (3 min) and ramped to 310° C (held 25 min) with 4° C min^{-1} . Compounds
5 were assigned comparing mass spectra and retention times to published data. Concentrations
6 were determined by comparison with peak areas of squalane (F2 and F3) and *n*-eicosane-D42
7 (F1) as internal standards.

8 Compound specific stable carbon isotope ratios of biomarkers in F2 and F3.3 were measured
9 (2x) using a Thermo Trace GC gas chromatograph coupled to a Thermo Delta Plus isotope
10 ratio mass spectrometer. The GC was operated under the same conditions and with the same
11 column as for GC-MS. The combustion reactor contained CuO, Ni and Pt and was operated at
12 940° C. Isotopic compositions are reported in standard delta notation relative to the Vienna
13 PeeDee Belemnite (V-PDB) and were calculated by comparison with an isotopically known
14 CO₂ reference gas. GC-C-IRMS precision and linearity was checked daily using an external
15 *n*-alkane isotopic standard (provided by A. Schimmelmann, Indiana University).

16

17 **2.5 Liquid chromatography-mass spectrometry (LC-MS)**

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

33

34 **2.6 Principle Component Analysis (PCA)**

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

4

5 **3 Results**

6 **3.1 Physicochemical parameters of the water column**

7 In summer 2011, the Landsort Deep showed a strong vertical stratification (Fig. 2). The oxic
8 zone consisted of the uppermost 80 m and was divided by a strong thermocline into a warm
9 surface layer (~0-10 m) and a cold winter water layer (~10-60 m). The halocline was located
10 between 60 m and 80 m. O₂ concentrations rapidly decreased from >8 ml l⁻¹ at ~50 m to <0.2
11 ml l⁻¹ at ~80 m, defining the upper boundary of the suboxic zone (Tyson and Pearson, 1991).
12 H₂S was first detected at 83 m. Because O₂ concentrations could methodically only be
13 measured in the complete absence of H₂S, oxygen could not be traced below this depth.
14 Therefore, the lower boundary of the suboxic zone was defined to be at 90 m, where H₂S
15 concentrations were sharply increasing. The upper suboxic zone also showed a sharp peak in
16 turbidity that is possibly caused by precipitation of Fe and Mn oxides (Dellwig et al., 2010) or
17 zero-valent sulfur (Kamyshny Jr. et al., 2013) and can be used as an indicator for the O₂-H₂S
18 transition (Kamyshny Jr. et al., 2013). The anoxic zone extends from 90 m to the bottom and
19 is characterized by the complete absence of O₂ and high concentrations of H₂S and CH₄.
20 CH₄ was highest in the deep anoxic zone, decreased strongly towards the suboxic zone but
21 was still present in minor concentrations in the oxic zone. A small CH₄ peak was detected at
22 the suboxic-anoxic interface (Fig. 2). Particulate organic carbon (POC) was highest at 10 m
23 (380 µg l⁻¹), decreased to a minimum in the cold winter water layer (48 µg l⁻¹) and showed
24 almost constant values of ~70 µg l⁻¹ in the suboxic and anoxic zones.

25 Generally, we follow the zonation of the Landsort Deep water column as given in Jakobs et
26 al. (2014). We regarded the onset of H₂S as the top of the anoxic zone, however, as this is
27 better supported by our biomarker data (see below).

28

29 **3.2 Lipid analysis**

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

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

6 7 **3.2.1 Group 1: surface maximum**

8 The first group is defined by a strong maximum in the surface layer and only minor
9 concentrations in greater depths. A subgroup of 14 compounds exclusively occurs at 10 m
10 water depth (Fig. 3). For the other compounds, abundance in greater water depths increases
11 towards the *y*-axis. 7-methylheptadecane (52), 24-ethylcholest-5-en-3 β -ol (β -sitosterol; 48),
12 20:4 ω 6 PLFA (34), 20:5 ω 3 PLFA (33), 16:1 ω 7c PLFA (11), and cholest-5-en-3 β -ol
13 (cholesterol; 44) were taken as representative for group 1. Among these compounds, 16:1 ω 7
14 PLFA and cholesterol showed the highest concentrations (1154 ng l⁻¹ and 594 ng l⁻¹,
15 respectively), and 7-methylheptadecane the lowest (6 ng l⁻¹, Fig. 4). Apart from their
16 maximum in the surface layer, the fate of these biomarkers in deeper water layers differed. 7-
17 methylheptadecane exclusively occurred in the surface layer, whereas 20:4 ω 6 was traceable
18 throughout the water column. β -sitosterol occurred in the surface and the bottom layers.
19 Unlike the other compounds, cholesterol and 20:5 ω 3 PLFA did not show a straight decrease
20 with depth, rather are there minor occurrences right above and at the bottom of the suboxic
21 zone, respectively. $\delta^{13}\text{C}$ values of all compounds were between -32 and -26 ‰ (Table 1).

22 23 **3.2.2 Group 2: surface and lower suboxic zone maxima**

24 Group 2 shows a surface maximum like group 1, but exhibits a stronger emphasis of the lower
25 suboxic zone (Fig. 4). With the exception of 16:7 ω 7t, all compounds were chosen for further
26 consideration. 4 α ,23,24-trimethyl-5 α -cholest-22E-en-3 β -ol (dinosterol; 49) and gammacer-
27 3 β -ol (tetrahymanol; 50) had their maximum concentration in the surface water (dinosterol:
28 66 ng l⁻¹; tetrahymanol: 42 ng l⁻¹) and were not detectable in the layers below, until a sharp
29 second maximum occurred at the bottom of the suboxic zone. Concentrations decreased again
30 below the suboxic zone and remained constantly low in the bottom water. Unlike these
31 compounds, *ai* 15:0 PLFA (5), total bacteriohopanepolyols (BHPs; 84), and the hopanoid
32 hydrocarbon hop-22(29)-ene (diploptene; 54) showed steadily increasing concentrations
33 through the suboxic zone and further increasing concentrations in the anoxic zone. The $\delta^{13}\text{C}$
34 values of all compounds were between -35 and -25 ‰ (Table 1).

1

2 **3.2.3 Group 3: cold winter water layer maximum**

3 The third group showed compounds that peaked in the cold winter water layer at 65 m water
4 depth (Fig. 3). 17:1 ω 9 PLFA (19) only occurred at 70 m water depth and *n*-C₂₁ (61) from 10
5 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
6 only occurred from 65 to 80 m, with a maximum at 65 m (287 ng l⁻¹ and 228 ng l⁻¹,
7 respectively). Out of group 3, the 16:0-18:1 wax ester was included into the discussion. $\delta^{13}\text{C}$
8 values of the wax esters were ~ -28‰ (Table 1).

9

10 **3.2.4 Group 4: oxic zone maximum**

11 Group 4 consisted exclusively of saturated *n*-alkanes from *n*-C₂₁ to *n*-C₃₆ as well as 26:0
12 PLFA (43). 26:0 PLFA only occurred at 80 m, whereas all other compounds were abundant
13 from the surface to the upper suboxic zone at 80 m (data not shown). The homologues *n*-C₂₇
14 (74), *n*-C₂₉ (76), and *n*-C₃₁ (78) show maxima at the surface (21 - 30 ng l⁻¹). For the other
15 compounds, maxima were either located at 65 or 70 m, with highest concentrations for *n*-C₂₅ -
16 *n*-C₃₆ (10 - 23 ng l⁻¹). Below 80 m, concentrations dropped to constantly low values. As an
17 example, the depth profile of *n*-C₂₅ (71) is shown in Figure 4. $\delta^{13}\text{C}$ values for these
18 compounds were not obtained.

19

20 **3.2.5 Group 5: suboxic zone maximum**

21 Group 5 contained only two compounds, 16:1 ω 8c PLFA (10) and the *n*-C_{26:1} alkene (72). *n*-
22 C_{26:1} occurred in very low concentrations at 10m, and peaked at 80 and 95 m (7-8 ng l⁻¹).
23 16:1 ω 8c PLFA occurred only at 80 and 90 m water depth, with highest values at 80 m (8 ng l⁻¹
24 ¹; Fig. 4), and was chosen for further discussion. $\delta^{13}\text{C}$ values of this compound were ~ -45‰
25 (Table 1).

26

27 **3.2.6 Group 6: absent in oxic zone, bottom layer maximum**

28 Group 6 consisted of compounds that only occurred in the suboxic zone and below, and
29 increased in concentration into the anoxic zone. An exception is 5 α (H)-cholestan-3 β -ol
30 (cholestanol; 45), which was also present in the surface layer. 10-me-16:0 PLFA (16), the
31 irregular C₂₅ isoprenoid 2,6,10,15,19-pentamethylcosane (PMI) and three unsaturated
32 derivatives thereof (PMI Δ ; 53), 2,3-di-*o*-isopranyl *sn*-glycerol diether (archaeol; 51), and
33 cholestanol were considered for further discussion. For all compounds, maxima were detected
34 in the anoxic zone, with highest concentrations observed for cholestanol (35 ng l⁻¹) followed

1 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
2 PLFA shows, compared to other compounds, a slight ¹³C depletion in the anoxic zone (-35.4
3 ‰; Table 1). Concentrations of archaeol, PMI, and PMI Δ were too low to determine δ¹³C.

5 **3.2.7 *n*-alkanes and *n*-alkenes in the sea surface layer**

6 The concentrations of *n*-alkanes and *n*-alkenes in the surface sample (10 m water depth) are
7 given in Fig. 5. The longest *n*-alkane chain was *n*-C₃₆, and odd carbon numbers dominated
8 over even. Highest concentrations were found for *n*-C₂₇ (21 ng l⁻¹), *n*-C₂₉ (30 ng l⁻¹), and *n*-C₃₁
9 (26 ng l⁻¹). The longest *n*-alkene chain was *n*-C_{26:1}, and highest *n*-alkene concentrations were
10 measured for *n*-C_{23:1} (3 ng l⁻¹) and *n*-C_{25:1} (3 ng l⁻¹).

12 **3.2.8 Water column profiles of BHPs**

13 In the Landsort Deep, seven individual BHPs were identified (Fig. 6a). In all samples,
14 bacteriohopane-32,33,34,35-tetrol (BHT) accounted for the greatest portion of the total BHPs
15 (88- 94%). An as yet uncharacterized BHT isomer, BHT II, was present only below 70 m and
16 showed its highest relative abundance (~2 %) between 70 and 90 m. BHT cyclitol ether, BHT
17 glucosamine, and 35-aminobacteriohopane-32,33,34-triol (aminotriol) were present
18 throughout the water column. BHT cyclitol ether and BHT glucosamine were most abundant
19 in the oxic zone (ca. 1-4%), but showed only minor abundances (< 1%) below. Aminotriol
20 was elevated at 65 and 420 m (~7 and ~5%, respectively). 35-aminobacteriohopane-
21 31,32,33,34-tetrol (aminotetrol) occurred throughout the suboxic and anoxic zones, whereas
22 35-aminobacteriohopane-30,31,32,33,34-pentol (aminopentol) was observed only at 90 m and
23 below. Both, aminotetrol and aminopentol showed minor relative abundances of ~2% and
24 <1% of the total BHPs, respectively (Jakobs et al. 2014).

25 For comparison, the major phytoplankton species from a cyanobacterial bloom in the Gotland
26 Deep (2012) were determined by microscopy (HELCOM manual, 2012) and the POM was
27 analysed for BHPs. This reference biomass contained mainly *Aphanizonemon* and, to a
28 smaller extent, *Anabaena* and *Nodularia*, which were accompanied by dinoflagellates. Three
29 BHPs were observed in the bloom POM (Fig. 6b). Among these compounds, the most
30 abundant was BHT (~86 %), followed by BHT cyclitol ether (~10%), and BHT glucosamine
31 (~4%).

33 **4 Discussion**

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

4 5 **4.1 Water column redox zones as reflected by cholestanol/cholesterol ratios**

6 Different redox states of the Landsort Deep water column and the associated microbial
7 processes are reflected by the profiles of cholesterol and its diagenetic product, cholestanol
8 (Fig. 4, groups 1 and 6, respectively). Cholesterol is synthesized by various eukaryotic phyto-
9 and zooplankton and higher plants (Parrish et al., 2000) and abundant in water columns and
10 sediments. In sediments as well as in stratified water columns, stanols are produced from
11 sterols by anaerobic bacterial hydrogenation (Gaskell and Eglinton, 1975; Wakeham, 1989)
12 and by the abiotic reduction of double bonds by reduced inorganic species such as H₂S
13 (Hebting et al., 2006; Wakeham et al., 2007). Therefore, cholestanol/cholesterol ratios
14 typically increase under more reducing conditions. In the Black Sea, low ratios of ~0.1 were
15 associated with oxygenated surface waters, the suboxic zone showed ratios between 0.1 and 1,
16 whereas the anoxic zone revealed values >1 (Wakeham et al., 2007). In the Landsort Deep,
17 the cholestanol/cholesterol ratios showed a slight increase with depth from the surface
18 towards the suboxic zone, but always remained <0.1 (Fig. 4). Below, the values increased to
19 ~0.3 in the suboxic zone, and further to a maximum of 0.45 in the anoxic zone. Whereas the
20 ratios in the Landsort Deep are considerably lower than in the Black Sea, the depth trend still
21 clearly mirrors the changes from oxic to suboxic, and further to anoxic conditions. It is also
22 interesting to note that total cholesterol and cholestanol concentrations in the Landsort Deep
23 were ten- and fourfold higher, respectively, as in the Black Sea (Wakeham et al., 2007).

24 25 **4.2 Phototrophic primary production**

26 As expected, *in situ* biomarkers for phototrophic organisms were most abundant in the surface
27 layer and are pooled in PCA group 1. 20:4 ω 6 PLFA is a biomarker traditionally assigned to
28 eukaryotic phytoplankton (Nanton and Castell, 1999; Lang et al., 2011) and organisms
29 grazing thereon, such as protozoa (Findlay and Dobbs, 1993; Pinkart et al., 2002; Risse-Buhl
30 et al., 2011). 20:5 ω 3 PLFA is known to be a major compound in diatoms (Arao and Marada,
31 1994; Dunstan et al., 1994) and high concentrations of these PLFAs, as observed in the
32 surface layer of the oxic zone, are in good agreement with such an autochthonous plankton-
33 based source.

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

13 Unlike the *n*-alkenes that only occurred in the surface layer, long-chain *n*-alkanes were
14 present in the whole water column, with high abundances in the oxic zone. Long-chain *n*-
15 alkanes with a strong predominance of the odd-numbered *n*-C₂₅ to *n*-C₃₆ homologues
16 (Eglinton and Hamilton, 1967; Bi et al., 2005) and β -sitosterol (Volkman, 1986) are typical
17 components of higher plant lipids, thus indicating continental runoff and/or aeolian input of
18 terrigenous OM into the Landsort Deep. *n*-C₂₇, *n*-C₂₉, and *n*-C₃₁ showed surface maxima (not
19 shown), indicating similar sources as for β -sitosterol and a contribution of land plant leaf
20 waxes. Other than β -sitosterol, most *n*-alkanes peaked between 65 and 70 m (*n*-C₂₅ for
21 example; Fig. 4). Apart from the surface peaks, this is also true for *n*-C₂₇, *n*-C₂₉, and *n*-C₃₁. A
22 possible explanation is the accumulation of terrigenous higher plant particles accumulating at
23 the pycnocline, where density differences were highest (MacIntyre et al., 1995)

24

25 **4.3 Phototrophic vs. heterotrophic dinoflagellates, and ciliates**

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

1 2007). However, *P. catenata* as well as *S. hangoei* are virtually absent below 50 m water
2 depth (Höglander et al., 2004) and can thus not account for the second peak of dinosterol at
3 the suboxic-anoxic transition zone. An accumulation of surface-derived dinosterol at the
4 bottom of the suboxic zone is unlikely, as the pycnocline and thus, the strongest density
5 discontinuity, is located at 60-70 m water depth, i.e. about 20 m above. Dinosterol is absent in
6 the pycnocline and only occurs from the bottom of the suboxic zone on and below. Instead, a
7 likely source of dinosterol at this water depth are heterotrophic dinoflagellates that are
8 abundant in the suboxic zones of the central Baltic Sea (Anderson et al., 2012). Due to their
9 enhanced productivity, these environments provide good conditions to sustain communities of
10 eukaryotic grazers (Detmer et al., 1993). A possible candidate, *Gymnodinium beii*, was
11 described from the suboxic zones of the central Baltic Sea (Stock et al., 2009). Indeed, several
12 *Gymnodinium* species are known to be heterotrophs (Strom and Morello, 1998) and some
13 have been reported to produce dinosterol (Mansour et al., 1999). Like cholesterol and β -
14 sitosterol, dinosterol was also found in the anoxic zone at 400 m water depth. The production
15 of these compounds at this depth is unlikely, as the synthesis of sterols requires oxygen
16 (Summons et al., 2006). Hence, the observed sterol occurrences probably reflect transport
17 through the water column.

18 A similar concentration distribution as for dinosterol was observed for tetrahymanol.
19 Tetrahymanol is known to be produced by ferns, fungi, and bacteria such as the purple non-
20 sulfur bacterium *Rhodopseudomonas palustris* (Zander et al., 1969; Kemp et al., 1984;
21 Kleemann et al., 1990; Sinninghe Damsté et al., 1995; Eickhoff et al., 2013). Moreover,
22 ciliates ubiquitously produce tetrahymanol as a substitute for cholesterol when grazing on
23 prokaryotes instead of eukaryotes such as algae (Conner et al., 1968; Boschker and
24 Middelburg, 2002). This is also a feasible scenario for the Baltic Sea where the ciliate genera
25 *Metopus*, *Strombidium*, *Metacystis*, *Mesodinium*, and *Coleps* are abundant in the suboxic zone
26 and at the suboxic-anoxic interface (Detmer et al., 1993; Anderson et al., 2012). Unidentified
27 ciliates also occurred in the anoxic waters of the Landsort Deep (Anderson et al., 2012).
28 Members of the genus *Rhodopseudomonas*, a possible alternative source of tetrahymanol,
29 have so far not been identified in the suboxic zone (Labrenz et al., 2007; Thureborn et al.,
30 2013). We therefore regard bacterivorous ciliates living under suboxic to anoxic conditions as
31 the most likely source of tetrahymanol in the suboxic zone and below. Likewise, ciliates
32 feeding on chemoautotrophic bacteria were assumed as producers of tetrahymanol in the
33 suboxic zone of the Black Sea (Wakeham et al., 2007). The situation is somewhat different in
34 the surface waters, where tetrahymanol shows its maximum concentrations at 10 m water

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

10 $\delta^{13}\text{C}$ values of tetrahymanol revealed an opposite trend as compared to dinosterol. While
11 dinosterol became isotopically more negative with depth (-29.9 to -32.0‰), tetrahymanol
12 became more positive (-28.7 to -25.9‰) and showed its highest $\delta^{13}\text{C}$ values in the anoxic
13 zone. Although ciliates and dinoflagellates are both grazers at the suboxic-anoxic interface,
14 they seem to occupy different ecological niches and feed on different bacterial sources.

15

16 **4.4 Heterotrophs in the cold winter water layer**

17 The only biomarkers with enhanced concentrations in the deep cold winter water layer are
18 wax esters (e.g. 16:0-18:1 wax ester, Fig. 4), and, to a minor extent, cholesterol and 20:5 ω 3
19 PLFA. As the pycnocline, and thus a strong density discontinuity, is also located at this depth,
20 an accumulation of settling organic debris containing these compounds has to be considered
21 (MacIntyre et al., 1995). Living organisms, however, may be also be plausible sources.

22 Known producers of wax esters and cholesterol are copepods (Lee et al., 1971; Sargent et al.,
23 1977; Kattner and Krause, 1989; Nanton and Castell, 1999; Falk-Petersen et al., 2002) which
24 are often abundant at density layers where they feed on accumulated aggregates (MacIntyre et
25 al., 1995). These organisms synthesize wax esters with total chain lengths between 28 and 44
26 carbon atoms (Lee et al., 1971; Kattner and Krause, 1989; Falk-Petersen et al., 2002), several
27 of which were present in the Landsort Deep (data not shown in Fig. 4), with roughly the same
28 distribution as the most prominent 16:0-18:1. Although copepods migrate through the water
29 column, particularly those rich in wax esters prefer deep water or near-surface cold water
30 (Sargent et al., 1977), which is in full agreement with the high amounts of these compounds in
31 the cold winter water layer. Copepods are abundant and diverse in the Baltic Sea, with major
32 species being *Pseudocalanus elongatus*, *Temora longicornis*, and *Acartia* spp. (Möllmann et
33 al., 2000; Möllmann and Köster, 2002). Like the wax esters, the 20:5 ω 3 PLFA shows higher
34 concentrations in the cold winter water layer, but it is also abundant in the surface and at the

1 suboxic-anoxic interface (Fig. 4). Copepods are also known to feed on diatoms and
2 incorporate their specific fatty acids such as 20:5 ω 3 PLFA largely unchanged into their own
3 tissues (Kattner and Krause, 1989). Dinoflagellates are also known producers of 20:5 ω 3
4 PLFA (Parrish et al., 1994; Volkman et al., 1998) and may be an alternative source in the
5 surface layer and at the suboxic-anoxic interface, which is supported by a good correlation
6 with dinosterol at these depths.

7 Unlike the abovementioned compounds, all other selected biomarkers show particularly low
8 concentrations in the cold winter water layer. This is also true for widespread compounds
9 such as the 16:1 ω 7c PLFA which is produced by eukaryotes (Pugh, 1971; Shamsudin, 1992)
10 as well as prokaryotes (Parkes and Taylor, 1983; Vestal and White, 1989). While a mixed
11 origin of 16:1 ω 7c PLFA has to be assumed for the oxic zone, a bacterial source is more
12 probable in the suboxic zone and in the anoxic zone. Regardless of the biological source, a
13 very low amount of this ubiquitous FA (Fig. 4) indicates that the cold winter water layer of
14 the Landsort Deep does not support abundant planktonic life. Based on microscopy, similar
15 observations have been made for the cold winter water layers of the Gotland, Bornholm and
16 Danzig Basins (Gast and Gocke, 1988, and citations therein).

17

18 **4.5 BHPs as indicators for aerobic and anaerobic metabolisms**

19 Bacteria are the only known source of BHPs (Kannenberg and Poralla, 1999). Although the
20 biosynthesis of BHPs and their precursor, diploptene (both plotting in group 2), does not
21 require oxygen, the production of hopanoids was long assumed to be restricted to aerobic
22 bacteria, as reports from facultatively or strictly anaerobic bacteria were initially missing.
23 More recently, however, planctomycetes (Sinninghe Damsté et al., 2004), metal reducing
24 *Geobacter* (Fischer et al., 2005), and sulfate reducing *Desulfovibrio* (Blumenberg et al., 2006;
25 2009; 2012) were identified as anaerobic producers of BHPs. In the Landsort Deep,
26 cyanobacteria are abundant in the surface water layer and may be considered as a major
27 source of BHPs (cf. Talbot et al., 2008; Welander et al., 2010). Evidence for such
28 cyanobacterial BHP contributions may come from our analysis of a Gotland Deep bloom from
29 summer 2012 (see 3.2.7). BHPs identified in this bloom were BHT, BHT cyclitol ether, and
30 BHT glucosamine (Fig. 6b) which is in line with the BHP composition of the Landsort Deep
31 surface layer (Fig. 6a). These three cyanobacterial BHPs were present throughout the
32 Landsort Deep water column, although they were minor in the suboxic zone and below. In
33 addition, the surface layer contained aminotriol that was also present in the whole water
34 column. Aminotriol is an abundant BHP produced by various bacteria (e.g. Talbot and

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

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

17 Like BHT II, aminotetrol and aminopentol are absent from the surface layer (Fig. 6 a).
18 Whereas both BHPs are biomarkers for methanotrophic bacteria, the latter typically occurs in
19 type I methanotrophs (Talbot et al., 2001). The presence of type I methanotrophic bacteria is
20 further supported by the co-occurrence of the specific 16:1 ω 8c PLFA (Nichols et al., 1985;
21 Bowman et al., 1991; Bowman et al., 1993) and its considerably depleted $\delta^{13}\text{C}$ value (-
22 45.4‰).

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

34

1 **4.6 Microbial processes in the anoxic zone**

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

13 In addition to the bacterial FA, two archaeal *in situ* biomarkers, archaeol and PMI, were
14 identified. Archaeol is the most common ether lipid in archaea, but is especially abundant in
15 euryarchaeotes, including methanogens (Tornabene and Langworthy, 1979; Koga et al.,
16 1993). Likewise, PMI and its unsaturated derivatives are diagnostic for methanogenic
17 euryarchaeotes (Tornabene et al., 1979; De Rosa and Gambacorta, 1988; Schouten et al.,
18 1997). In the Landsort Deep, both compounds are virtually absent in the oxic zone, and
19 increase in abundance with depth through the suboxic zone (Fig. 3). The same trend has been
20 described for PMI in the Black Sea (Wakeham et al., 2007) and the presence of euryarchaeota
21 in Landsort Deep anoxic waters has recently been proven by Thureborn et al. (2013).

22 Given the available sample resolution, it is impossible to further elucidate the exact
23 distribution of archaea in the anoxic zone of the Landsort Deep. Likewise, $\delta^{13}\text{C}$ values could
24 not be obtained for archaeol and PMI due to low compound concentrations, which excludes
25 statements on inputs of these lipids from archaea involved in the sulfate-dependent anaerobic
26 oxidation of methane (AOM; cf. Hinrichs et al., 1999; Thiel et al., 2001). Whereas it has been
27 shown that AOM is theoretically possible in the anoxic zone of the Landsort Deep and
28 anaerobic methane consumption has recently been demonstrated to occur (Jakobs et al.,
29 2013), a clear evidence for abundant AOM is as yet lacking and requires further investigations
30 focused at the anoxic water bodies of the Baltic Sea.

31 32 **5 Conclusions**

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

1 ecosystems and their distributions in the water column can be reconstructed using diverse *in*
2 *situ* biomarkers (Fig. 7). According to their behavior in the water column, PCA analysis
3 revealed six groups of biomarkers for distinct groups of (micro)organisms and the related
4 biogeochemical processes. Within the oxic zone, a clear preference for the surface layer
5 became obvious for distinctive biomarkers. Among these compounds, 7-methylheptadecane,
6 different alkenes, BHT cyclitol ether, and BHT glucosamine were indicative for the presence
7 of bacterial primary producers, namely cyanobacteria. Dinosterol concentrations and $-\delta^{13}\text{C}$
8 values revealed a phototrophic dinoflagellate population in the surface waters, and a second,
9 heterotrophic community thriving at the suboxic-anoxic interface. Similarly, abundant
10 tetrahymanol at the surface indicated ciliates feeding on cyanobacterial agglomerates, but a
11 second maximum at the suboxic-anoxic interface suggested a further ciliate population that
12 grazed on chemo-autotrophic bacteria. The cold winter water layer at the bottom of the oxic
13 zone showed only low concentrations of biomarkers and seemed to be avoided by most
14 organisms, except copepods. In contrast, biomarkers obtained from the suboxic zone reflected
15 a high abundance and diversity of eukaryotes and prokaryotes. Whereas 16:1 ω 8 PLFA and
16 aminopentol revealed the presence of type I aerobic methane oxidizing bacteria, *ai*-15:0
17 PLFA, 10-me-16:0 and total BHPs indicated the distribution of sulfate reducing bacteria in
18 the Landsort Deep water column. The close coupling of *ai*-15:0 PLFA with total BHPs
19 suggests that these bacteria represent a major *in situ* source for hopanoids in the anoxic zone.
20 The anoxic zone was further inhabited by most likely euryarchaeota, as shown by the
21 presence of archaeol and PMI and its derivatives. Our study in the water column of the
22 Landsort Deep gives insights into the recent distributions and actual sources of organic matter
23 as reflected by lipid biomarkers. The results may also aid in the interpretation of organic
24 matter preserved in the sedimentary record, and thus help to better constrain changes in the
25 geological history of the Baltic Sea.

26

27 **Acknowledgments**

28 We thank the Captains and crews of R/Vs *Elisabeth Mann Borghese* and *Meteor* for
29 assistance during the cruises. We thank C. Conradt and L. Kammel for laboratory assistance,
30 T. Licha and K. Nödler for help with LC-MS, and N. Cerveau for help with "R". We thank S.
31 Bühring and an anonymous reviewer for helpful comments on our manuscript. The German
32 Research Foundation (Deutsche Forschungsgemeinschaft, DFG) is acknowledged for
33 financial support (Grants BL 971/1-3 and 971/3-1).

34

1 **References**

- 2 Anderson, R., Winter, C., and Jürgens, K.: Protist grazing and viral lysis as prokaryotic
3 mortality factors at Baltic Sea oxic-anoxic interfaces, *Mar. Ecol. Progr.*, 467, 1-14, 2012.
- 4 Arao, T. and Yamada, M.: Biosynthesis of polyunsaturated fatty acids in the marine diatom,
5 *Phaeodactylum tricornutum*. *Phytochemistry*, 35, 1177-1181, 1994.
- 6 Beliaeff, B. and Burgeot, T.: Integrated biomarker response: a useful tool for ecological risk
7 assessment, *Environ. Toxicol. Chem.*, 21, 1316-1322, 2001.
- 8 Bergström, S. and Matthäus, W.: Meteorology, hydrology and hydrography, HELCOM,
9 Helsinki, 1996.
- 10 Berndmeyer, C., Thiel, V., Schmale, O., and Blumenberg, M.: Biomarkers for aerobic
11 methanotrophy in the water column of the stratified Gotland Deep (Baltic Sea), *Org.*
12 *Geochem.*, 55, 103-111, 2013.
- 13 Bi, X., Sheng, G., Liu, X., Li, C., and Fu, J.: Molecular and carbon and hydrogen isotopic
14 composition of *n*-alkanes in plant leaf waxes, *Org. Geochem.*, 36, 1405-1417, 2005.
- 15 Blumenberg, M., Krüger, M., Nauhaus, K., Talbot, H. M., Oppermann, B. I., Seifert, R., Pape,
16 T., and Michaelis, W.: Biosynthesis of hopanoids by sulfate-reducing bacteria (genus
17 *Desulfovibrio*), *Environ. Microbiol.*, 8, 1220-1227, 2006.
- 18 Blumenberg, M., Seifert, R., and Michaelis, W.: Aerobic methanotrophy in the oxic–anoxic
19 transition zone of the Black Sea water column, *Org. Geochem.*, 38, 84-91, 2007.
- 20 Blumenberg, M., Oppermann, B. I., Guyoneaud, R., and Michaelis, W.: Hopanoid production
21 by *Desulfovibrio bastinii* isolated from oilfield formation water, *FEMS Microbiol. Lett.*, 293,
22 73-78, 2009.
- 23 Blumenberg, M., Hoppert, M., Krüger, M., Dreier, A., and Thiel, V.: Novel findings on
24 hopanoid occurrences among sulfate reducing bacteria: Is there a direct link to nitrogen
25 fixation?, *Org. Geochem.*, 49, 1-5, 2012.
- 26 Blumenberg, M., Berndmeyer, C., Moros, M., Muschalla, M., Schmale, O., and Thiel, V.:
27 Bacteriohopanepolyols record stratification, nitrogen fixation and other biogeochemical

- 1 perturbations in Holocene sediments of the central Baltic Sea, *Biogeosciences*, 10, 2725-2735,
2 2013.
- 3 Boon, J. J., Rijpstra, W. I. C., De Lange, F., and De Leeuw, J. W.: Black Sea sterol - a
4 molecular fossil for dinoflagellate blooms, *Nature*, 277, 125-127, 1979.
- 5 Boschker, H. T. S. and Middelburg, J. J.: Stable isotopes and biomarkers in microbial
6 ecology, *FEMS Microbiol. Ecol.*, 40, 85-95, 2002.
- 7 Bowman, J. P., Skeratt, J. H., Nichols, P. D., and Sly, L. I.: Phospholipid fatty acid and
8 lipopolysaccharide fatty acid signature lipids in methane-utilizing bacteria, *FEMS Microbiol.*
9 *Ecol.*, 85, 15-22, 1991.
- 10 Bowman, J. P., Sly, L. I., Nichols, P. D., and Hayward, A. C.: Revised taxonomy of the
11 methanotrophs: Description of *Methylobacter* gen. nov., emendation of *Methylococcus*,
12 validation of *Methylosinus* and *Methylocystis* species, and a proposal that the family
13 *Methylococcaceae* includes only the group I methanotrophs, *Int. J. Syst. Bacteriol.*, 43, 735-
14 753, 1993.
- 15 Carlson, D. R., Roan, C.-S., Yost, R. A., and Hector, J.: Dimethyl disulfide derivatives of
16 long chain alkenes, alkadienes, and alkatrienes for gas chromatography / mass spectrometry,
17 *Anal. Chem.*, 61, 1564-1571, 1989.
- 18 Conner, R. L., Landrey, J. R., Burns, C. H., and Mallory, F. B.: Cholesterol inhibition of
19 pentacyclic triterpenoid biosynthesis in *Tetrahymena pyriformis*, *J. Protozool.*, 15, 600-605,
20 1968.
- 21 De Rosa, M. and Gambacorta, A.: The lipids of archaebacteria, *Prog. Lip. Res.*, 27, 153-175,
22 1988.
- 23 Dellwig, O., Leipe, T., März, C., Glockzin, M., Pollehne, F., Schnetger, B., Yakushev, E. V.,
24 Böttcher, M. E., and Brumsack, H.-J.: A new particulate Mn-Fe-P-shuttle at the redoxcline of
25 anoxic basins, *Geochim. Cosmochim. Acta*, 74, 7100-7115, 2010.
- 26 Detmer, A. E., Giesenhausen, H. C., Trenkel, V. M., Auf dem Venne, H., and Jochem, F.:
27 Phototrophic and heterotrophic pico- and nanoplankton in anoxic depths of the central Baltic
28 Sea, *Mar. Ecol. Progr.*, 99, 197-203, 1993.

- 1 Dunstan, G. A., Volkman, J. K., Barrett, S. M., Leroi, J.-M., and Jeffrey, S. W.: Essential
2 polyunsaturated fatty acids from 14 species of diatom (Bacillariophyceae). *Phytochemistry*,
3 35, 155-161, 1994.
- 4 Eglinton, G. and Hamilton, R. J.: Leaf epicuticular waxes, *Science*, 156, 1322-1335, 1967.
- 5 Eickhoff, M., Birgel, D., Talbot, H. M., Peckmann, J., and Kappler, A.: Oxidation of Fe(II)
6 leads to increased C-2 methylation of pentacyclic triterpenoids in the anoxygenic phototrophic
7 bacterium *Rhodospseudomonas palustris* strain TIE-1. *Geobiology*, 11,268-278, 2013.
- 8 Falk-Petersen, S., Dahl, T. M., Scott, C. L., Sargent, J. R., Gulliksen, B., Kwasniewski, S.,
9 Hop, H., and Millar, R.-M.: Lipid biomarkers and trophic linkages between ctenophores and
10 copepods in Svalbard waters, *Mar. Ecol. Progr.*, 227, 187-194, 2002.
- 11 Farnelid, H., Öberg, T., and Riemann, L.: Identity and dynamics of putative N₂-fixing
12 picoplankton in the Baltic proper suggest complex patterns of regulation. *Environmental*
13 *Microbiology Reports*, 1, 145-154, 2009.
- 14 Findlay, R. H. and Dobbs, F. C.: Quantitative description of microbial communities using
15 lipid analysis, in: *Handbook of methods in aquatic microbial ecology*, edited by: Cole, J. J.,
16 CRC Press, Florida, USA, 1993.
- 17 Fischer, W. W., Summons, R. E., and Pearson, A.: Targeted genomic detection of
18 biosynthetic pathways: anaerobic production of hopanoid biomarkers by a common
19 sedimentary microbe, *Geobiology*, 3, 33-40, 2005.
- 20 Gaskell, S. J. and Eglinton, G.: Rapid hydrogenation of sterols in a contemporary lacustrine
21 sediment, *Nature*, 254, 209-211, 1975.
- 22 Gast, V. and Gocke, K.: Vertical distribution of number, biomass and size-class spectrum of
23 bacteria in relation to oxic/anoxic conditions in the Central Baltic Sea, *Mar. Ecol. Progr.*, 45,
24 179-186, 1988.
- 25 Gatellier, J.-P. L. A., de Leeuw, J. W., Sinninghe Damsté, J. S., Derenne, S., Largeau, C., and
26 Metzger, P.: A comparative study of macromolecular substances of a Coorongite and cell
27 walls of the extant alga *Botryococcus braunii*, *Geochim. Cosmochim. Acta*, 57, 2053-2068,
28 1993.

- 1 Gelpi, E., Schneider, H., Mann, J., and Oró, J.: Hydrocarbons of geochemical significance in
2 microscopic algae, *Phytochemistry*, 9, 603-612, 1970.
- 3 Grasshoff, K., Kremling, K., Ehrhardt, M.: *Methods of seawater analysis*. Verlag Chemie,
4 Weinheim, Germany, 1983.
- 5 Hajdu, S., Högländer, H., and Larsson, U.: Phytoplankton vertical distributions and
6 compositions in Baltic Sea cyanobacterial blooms, *Harmful Algae*, 6, 189-205, 2007.
- 7 Hanson, N., Förlin, L., and Larsson, Å.: Evaluation of long-term biomarker data from perch
8 (*Perca fluviatilis*) in the Baltic Sea suggests increasing exposure to environmental pollutants,
9 *Environ. Toxicol. Chem.*, 28, 364-373, 2009.
- 10 Hebbing, Y., Schaeffer, P., Behrens, A., Adam, P., Schmitt, G., Schneckeburger, P.,
11 Bernasconi, S. M., and Albrecht, P.: Biomarker evidence for a major preservation pathway of
12 sedimentary organic carbon, *Science*, 312, 1627-1631, 2006.
- 13 HELCOM COMBINE: Manual for marine monitoring in the COMBINE programme of
14 HELCOM, Part C, Programme for monitoring of eutrophication and its effects, Annex C-6,
15 Guidelines concerning phytoplankton species composition, abundance and biomass.
16 HELCOM, 2012.
- 17 Hietanen, S., Jäntti, H., Buizert, C., Jürgens, K., Labrenz, M., Voss, M., and Kuparinen, J.:
18 Hypoxia and nitrogen processing in the Baltic Sea water column, *Limnol. Oceanogr.* 57, 325-
19 337, 2012.
- 20 Hinrichs, K. U., Hayes, J. M., Sylva, S. P., Brewer, P. G., and DeLong, E. F.: Methane-
21 consuming archaeobacteria in marine sediments, *Nature*, 398, 802-805, 1999.
- 22 Högländer, H., Larsson, U., and Hajdu, S.: Vertical distribution and settling of spring
23 phytoplankton in the offshore NW Baltic Sea proper, *Mar. Ecol. Progr.*, 283, 15-27, 2004.
- 24 Jakobs, G., Rehder, G., Jost, G., Kießlich, K., Labrenz, M., and Schmale, O.: Comparative
25 studies of pelagic microbial methane oxidation within the redox zones of the Gotland Deep
26 and Landsort Deep (central Baltic Sea), *Biogeosciences*, 10, 7863-7875, 2013.
- 27 Jakobs, G., Holtermann, P., Berndmeyer, C., Rehder, G., Blumenberg, M., Jost, G., Nausch,
28 G., and schmale, O.: Seasonal and spatial methane dynamic in the water column of the central
29 Baltic Sea (Gotland Sea), *Cont. Shelf Res.*, 91, 12-25, 2014.

- 1 Kamyshny Jr., A., Yakushev, E. V., Jost, G., and Podymov, O. I.: Role of sulfide oxidation
2 intermediates in the redox balance of the oxic-anoxic interface of the Gotland Deep, Baltic
3 Sea, in: The handbook of environmental chemistry, edited by: Yakushev, E. V., Springer,
4 Berlin Heidelberg, 95-119, 2013.
- 5 Kannenberg, E. L. and Poralla, K.: Hopanoid biosynthesis and function in bacteria,
6 *Naturwissenschaften*, 86, 168-176, 1999.
- 7 Kattner, G. and Krause, M.: Seasonal variations of lipids (wax esters, fatty acids and alcohols)
8 in calanoid copepods from the North Sea, *Mar. Chem.*, 26, 261-275, 1989.
- 9 Kemp, P., Lander, D. J., and Orpin, C. G.: The lipids of the rumen fungus *Piromonas*
10 *communis*. *J. Gen. Microbiol.*, 130, 27-37, 1984.
- 11 Kleemann, G., Poralla, K., Englert, G., Kjösen, H., Liaaen-Jensen, S., Neunlist, S., and
12 Rohmer, M.: Tetrahymanol from the phototrophic bacterium *Rhodopseudomonas palustris*:
13 first report of a gammacerane triterpene from a prokaryote. *J. Gen. Microbiol.*, 136, 2551-
14 2553, 1990.
- 15 Koga, Y., Nishihara, M., Morii, H., and Akagawa-Matsushita, M.: Ether polar lipids of
16 methanogenic bacteria: structures, comparative aspects, and biosynthesis, *Microbiol. Revs.*,
17 57, 164-182, 1993.
- 18 Kononen, K., Kuparinen, J., Mäkelä, K., Laanemets, J., Pavelson, J., and Nömmann, S.:
19 Initiation of cyanobacterial blooms in a frontal region at the entrance to the Gulf of Finland,
20 Baltic Sea, *Limnol. Oceanogr.*, 41, 98-112, 1996.
- 21 Köster, J., Volkman, J. K., Rullkötter, J., Scholz-Böttcher, B. M., Rethmeier, J., and Fischer,
22 U.: Mono-, di- and trimethyl-branched alkanes in cultures of the filamentous cyanobacterium
23 *Calothrix scopulorum*, *Org. Geochem.*, 30, 1367-1379, 1999.
- 24 Kuever, J., Könnecke, M., Galushko, A., and Drzyzga, O.: Reclassification of
25 *Desulfobacterium phenolicum* as *Desulfobacula phenolica* comb. nov. and description of
26 strain Sax^T as *Desulfotignum balticum* gen. nov., sp. nov., *Int. J. Syst. Evol. Microbiol.*, 51,
27 171-177, 2001.

- 1 Labrenz, M., Jost, G., and Jürgens, K.: Distribution of abundant prokaryotic organisms in the
2 water column of the central Baltic Sea with an oxic-anoxic interface, *Aquat. Microb. Ecol.*,
3 46, 177-190, 2007.
- 4 Lang, I., Hodac, L., Friedl, T., and Feussner, I.: Fatty acid profiles and their distribution
5 patterns in microalgae: a comprehensive analysis of more than 2000 strains from the SAG
6 culture collection, *BMC Plant Biology*, 11, 124-140, 2011.
- 7 Leblond, J. D., Anderson, B., Kofink, D., Logares, R., Rengefors, K., and Kremp, A.: Fatty
8 acid and sterol composition of two evolutionary closely related dinoflagellate morphospecies
9 from cold Scandinavian brackish and freshwaters, *Eur. J. Phycol.*, 41, 303-311, 2007.
- 10 Lee, R. F., Nevenzel, J. C., and Paffenhöfer, G.-A.: Importance of wax esters and other lipids
11 in the marine food chain: phytoplankton and copepods, *Mar. Biol.*, 9, 99-108, 1971.
- 12 Lehtonen, K. K., Schiedek, D., Köhler, A., Lang, T., Vuorinen, P. J., Förlin, L., Baršienė, J.,
13 Pempkowiak, J., and Gercken, J.: The BEEP project in the Baltic Sea: Overview of results
14 and outline for a regional biological effects monitoring strategy, *Mar. Pollut. Bull.*, 53, 523-
15 537, 2006.
- 16 Liu, A., Zhu, T., Lu, X., and Song, L.: Hydrocarbon profiles and phylogenetic analyses of
17 diversified cyanobacterial species, *Appl. Energ.*, 111, 383-393, 2013.
- 18 MacIntyre, S., Alldredge, A. L., and Gotschalk, C. C.: Accumulation of marine snow at
19 density discontinuities in the water column. *Limnol. Oceanogr.*, 40, 449-468, 1995.
- 20 Mansour, M. P., Volkman, J. K., Jackson, A. E., and Blackburn, S. I.: The fatty acid and
21 sterol composition of five marine dinoflagellates, *J. Phycol.*, 35, 710-720, 1999.
- 22 Matsumoto, G. I., Akiyama, M., Watanuki, K., and Torii, T.: Unusual distribution of long-
23 chain *n*-alkanes and *n*-alkenes in Antarctic soil, *Org. Geochem.*, 15, 403-412, 1990.
- 24 Matthäus, W. and Schinke, H.: The influence of river runoff on deep water conditions of the
25 Baltic Sea, *Hydrobiologia*, 393, 1-10, 1999.
- 26 Möllmann, C., Kornilovs, G., and Sidrevics, L.: Long-term dynamics of main
27 mesozooplankton species in the central Baltic Sea, *J. Plankton. Res.*, 22, 2015-2038, 2000.

- 1 Möllmann, C. and Köster, F. W.: Population dynamics of calanoid copepods and the
2 implications of their predation by clupeid fish in the Central Baltic Sea, *J. Plankton. Res.*, 24,
3 959-977, 2002.
- 4 Nanton, D. A. and Castell, J. D.: The effects of temperature and dietary fatty acids on the fatty
5 acid composition of harpacticoid copepods, for use as a live food for fish larvae, *Aquaculture*,
6 175, 167-181, 1999.
- 7 Nichols, P. D., Smith, G. A., Antworth, C. P., Hanson, R. S., and White, D. C.: Phospholipid
8 and lipopolysaccharide normal and hydroxy fatty acids as potential signatures for methane-
9 oxidizing bacteria, *FEMS Microbiol. Ecol.*, 0, 327-335, 1985.
- 10 Parkes, R. J. and Taylor, J.: The relationship between fatty acid distributions and bacterial
11 respiratory types in contemporary marine sediments, *Estuar. Coast. Shelf Science*, 16, 173-
12 189, 1983.
- 13 Parrish, C. C., Bodennec, G., and Gentien, P.: Time courses of intracellular and extracellular
14 lipid classes in batch cultures of the toxic dinoflagellate, *Gymnodinium cf. nagasakiense*, *Mar.*
15 *Chem.*, 48, 71-82, 1994.
- 16 Parrish, C. C., Abrajano, T. A., Budge, S. M., Helleur, R. J., Hudson, E. D., Pulchan, K., and
17 Ramos, C.: Lipid and Phenolic Biomarkers in Marine Ecosystems: Analysis and Applications,
18 in: *Mar. Chem.*, edited by: Wangersky, P. J., *The Handbook of Environmental Chemistry*,
19 Springer Berlin Heidelberg, 193-223, 2000.
- 20 Pinkart, H. C., Ringelberg, D. B., Piceno, Y. M., Macnaughton, S. J., and White, D. C.:
21 Biochemical approaches to biomass measurements and community structure analysis, in:
22 *Manual of environmental microbiology*, 2nd ed., edited by: Hurt, C. J., ASM Press,
23 Washington, DC, 101-113, 2002.
- 24 Pugh, P. R.: Changes in the fatty acid composition of *Coscinodiscus eccentricus* with cultur-
25 age and salinity, *Mar. Biol.*, 11, 118-124, 1971.
- 26 Reissmann, J. H., Burchard, H., Feistel, R., Hagen, E., Lass, H. U., Mohrholz, V., Nausch, G.,
27 Umlauf, L., and Wieczorek, G.: State-of-the-art review on vertical mixing in the Baltic Sea
28 and consequences for eutrophication, *Progr. Oceanogr.*, 82, 47-80, 2009.

1 Risse-Buhl, U., Trefzger, N., Seifert, A.-G., Schönborn, W., Gleixner, G., and Küsel, K.:
2 Tracking the autochthonous carbon transfer in stream biofilm food webs, *FEMS Microbiol.*
3 *Ecol.*, 79, 118-131, 2011.

4 Rush, D., Sinninghe Damsté, J. S., Poulton, S. W., Thamdrup, B., Garside, A. L., González, J.
5 A., Schouten, S., Jetten, M. S. M., Talbot, H. M.: Anaerobic ammonium-oxidising bacteria: a
6 biological source of the bacteriohopanetetrol stereoisomer in marine sediments, *Geochim.*
7 *Cosmochim. Acta*, 140, 50-64, 2014.

8 Sáenz, J. P., Wakeham, S. G., Eglinton, T. I., and Summons, R. E.: New constraints on the
9 provenance of hopanoids in the marine geologic record: Bacteriohopanepolyols in marine
10 suboxic and anoxic environments, *Org. Geochem.*, 42, 1351-1362, 2011.

11 Sargent, J. R., Gatten, R. R., and McIntosh, R.: Wax esters in the marine environment - their
12 occurrence, formation, transformation and ultimate fates, *Mar. Chem.*, 5, 573-584, 1977.

13 Schmale, O., Blumenberg, M., Kießlich, K., Jakobs, G., Berndmeyer, C., Labrenz, M., Thiel,
14 V., and Rehder, G.: Microbial methane oxidation at the redoxcline of the Gotland Deep
15 (Baltic Sea), *Biogeosciences*, 9, 4969-4977, 2012.

16 Schneider, B., Nausch, G., Kubsch, H., and Petersohn, I.: Accumulation of total CO₂ during
17 stagnation in the Baltic Sea deep water and its relationship to nutrient and oxygen
18 concentrations, *Mar. Chem.*, 77, 277-291, 2002.

19 Schouten, S., Van der Maarel, M. J. E. C., Huber, R., and Sinninghe Damsté, J. S.:
20 2,6,10,15,19-Pentamethylcosenes in *Methanobolus bombayensis*, a marine methanogenic
21 archaeon, and in *Methanosarcina mazei*, *Org. Geochem.*, 26, 409-414, 1997.

22 Schouten, S., Wakeham, S. G., and Sinninghe Damsté, J. S.: Evidence for anaerobic methane
23 oxidation by archaea in euxinic waters of the Black Sea, *Org. Geochem.*, 32, 1277-1281,
24 2001.

25 Schubert, C. J., Coolen, M. J., Neretin, L. N., Schippers, A., Abbas, B., Durisch-Kaiser, E.,
26 Wehrli, B., Hopmans, E. C., Damste, J. S., Wakeham, S., and Kuypers, M. M.: Aerobic and
27 anaerobic methanotrophs in the Black Sea water column, *Environ. Microbiol.*, 8, 1844-1856,
28 2006.

- 1 Shamsudin, L.: Lipid and fatty acid composition of microalgae used in Malaysian aquaculture
2 as live food for the early stage of penaeid larvae, *J. Appl. Phycol.*, 4, 371-378, 1992.
- 3 Shiea, J., Brassel, S. C., and Ward, D. M.: Mid-chain branched mono- and dimethyl alkanes in
4 hot spring cyanobacterial mats: A direct biogenic source for branched alkanes in ancient
5 sediments?, *Org. Geochem.*, 15, 223-231, 1990.
- 6 Sinninghe Damsté, J. S., Kenig, F., Koopmans, M. P., Köster, J., Schouten, S., Hayes, J. M.,
7 and de Leeuw, J. W.: Evidence for gammacerane as an indicator of water column
8 stratification. *Geochim. Cosmochim. Acta*, 59, 1895-1900, 1995.
- 9 Sinninghe Damsté, J. S., Rijpstra, W. I. C., Schouten, S., Fuerst, J. A., Jetten, M. S. M., and
10 Strous, M.: The occurrence of hopanoids in planctomycetes: implications for the sedimentary
11 biomarker record, *Org. Geochem.*, 35, 561-566, 2004.
- 12 Sinninghe Damsté, J. S., Rijpstra, Geenevasen, J. A. J., Strous, M., Jetten, M. S. M.:
13 Structural identification of ladderane and other membrane lipids of planctomycetes capable of
14 anaerobic ammonium oxidation (anammox). *FEBS J.*, 272, 4270-4283, 2005.
- 15 Stal, L. J., Albertano, P., Bergmann, B., von Bröckel, K., Gallon, J. R., Hayes, P. K., Sivonen,
16 K., and Walsby, A. E.: BASIC: Baltic Sea cyanobacteria. An investigation of the structure
17 and dynamics of water blooms of cyanobacteria in the Baltic Sea - responses to a changing
18 environment, *Cont. Shelf Res.*, 23, 1695-1714, 2003.
- 19 Stock, A., Jürgens, K., Bunge, J., and Stoeck, T.: Protistan diversity in suboxic and anoxic
20 waters of the Gotland Deep (Baltic Sea) as revealed by 18S rRNA clone libraries, *Aquat.*
21 *Microb. Ecol.*, 55, 267-284, 2009.
- 22 Strom, S. L. and Morello, T. A.: Comparative growth rates and yields of ciliates and
23 heterotrophic dinoflagellates, *J. Plankton. Res.*, 20, 571-584, 1998.
- 24 Summons, R., Bradley, A. S., Jahnke, L. L., and Waldbauer, J. R.: Steroids, triterpenoids and
25 molecular oxygen. *Phil. Trans. R. Soc. B*, 361, 951-968, 2006.
- 26 Sturt, H. F., Summons, R. E., Smith, K., Elvert, M., and Hinrichs, K. U.: Intact polar
27 membrane lipids in prokaryotes and sediments deciphered by high-performance liquid
28 chromatography/electrospray ionization multistage mass spectrometry - new biomarkers for
29 biogeochemistry and microbial ecology, *Rapid Commun. Mass Sp.*, 18, 617-628, 2004.

- 1 Szewzyk, R. and Pfennig, N.: Complete oxidation of catechol by the strictly anaerobic sulfate-
2 reducing *Desulfobacterium catecholicum* sp. nov., Arch. Microbiol., 147, 163-168, 1987.
- 3 Talbot, H. M., Watson, D. F., Murrel, J. C., Carter, J. F., and Farrimond, P.: Analysis of intact
4 bacteriohopanepolyols from methanotrophic bacteria by reversed-phase high-performance
5 liquid chromatography-atmospheric pressure chemical ionisation mass spectrometry, J.
6 Chrom., 921, 175-185, 2001.
- 7 Talbot, H. M. and Farrimond, P.: Bacterial populations recorded in diverse sedimentary
8 biohopanoid distributions, Org. Geochem., 38, 1212-1225, 2007.
- 9 Talbot, H. M., Summons, R. E., Jahnke, L. L., Cockell, C. S., Rohmer, M., and Farrimond, P.:
10 Cyanobacterial bacteriohopanepolyol signatures from cultures and natural environmental
11 settings, Org. Geochem., 39, 232-263, 2008.
- 12 Taylor, J. and Parkes, J.: The cellular fatty acids of the sulphate-reducing bacteria,
13 *Desulfobacter* sp., *Desulfobulbus* sp. and *Desulfovibrio desulfuricans*, J. Gen. Microbiol.,
14 129, 3303-3309, 1983.
- 15 The R Foundation For Statistical Computing. R: A language and environment for statistical
16 computing. R website, available at <http://www.r-project.org/>. Accessed 2014 September 15,
17 2014.
- 18 Thiel, V., Peckmann, J., Richnow, H. H., Luth, U., Reitner, J., and Michaelis, W.: Molecular
19 signals for anaerobic methane oxidation in Black Sea seep carbonates and a microbial mat.
20 Mar. Chem. 73, 97-112, 2001
- 21 Thureborn, P., Lundin, D., Plathan, J., Poole, A. M., Sjöberg, B.-M., and Sjöling, S: a
22 metagenomics transect into the deepest point of the Baltic Sea reveals clear stratification of
23 microbial functional capacities, PLOS One 8, e74983, 2013.
- 24 Tornabene, T. G. and Langworthy, T. A.: Diphytanyl and dibiphytanyl glycerol ether lipids of
25 methanogenic archaeobacteria, Science, 203, 51-53, 1979.
- 26 Tornabene, T. G., Langworthy, T. A., Holzer, G., and Orò, J.: Squalenes, phytanes and other
27 isoprenoids as major neutral lipids of methanogenic and thermoacidophilic "archaeobacteria",
28 J. Mol. Evol., 13, 73-83, 1979.

- 1 Tyson, R. V. and Pearson, T. H.: Modern and ancient continental shelf anoxia: an overview,
2 Geological Society, London, Special Publications, 58, 1-24, 1991.
- 3 Vahtera, E., Conley, D. J., Gustafsson, B. G., Kuosa, H., Pitkänen, H., Savchuk, O. P.,
4 Tamminen, T., Viitasalo, M., Voss, M., Wasmund, N., and Wulff, F.: Internal ecosystem
5 feedbacks enhance nitrogen-fixing cyanobacteria blooms and complicate management in the
6 Baltic Sea, *AMBIO*, 36, 186-194, 2007.
- 7 Vainshtein, M., Hippe, H., and Kroppenstedt, R. M.: Cellular fatty acid composition of
8 *Desulfovibrio* species and its use in classification of sulfate-reducing bacteria, *Syst. Appl.*
9 *Microbiol.*, 15, 554-556, 1992.
- 10 Vestal, R. J. and White, D. C.: Lipid analysis in microbial ecology, *BioScience*, 39, 535-541,
11 1989.
- 12 Volkman, J. K.: A review of sterol markers for marine and terrigenous organic matter, *Org.*
13 *Geochem.*, 9, 83-99, 1986.
- 14 Volkman, J. K., Barrett, S. M., Dunstan, G. A., and Jeffrey, S. W.: Geochemical significance
15 of the occurrence of dinosterol and other 4-methyl sterols in a marine diatom, *Org. Geochem.*,
16 20, 7-15, 1993.
- 17 Volkman, J. K., Barrett, S. M., Blackburn, S. I., Mansour, M. P., Sikes, E. L., and Gelin, F.:
18 Microalgal biomarkers: A review of recent research developments, *Org. Geochem.*, 29, 1163-
19 1179, 1998.
- 20 Wakeham, S. G.: Reduction of stenols to stanols in particulate matter at oxic-anoxic
21 boundaries in sea water, *Nature*, 342, 787-790, 1989.
- 22 Wakeham, S. G., Amann, R., Freeman, K. H., Hopmans, E. C., Jørgensen, B. B., Putnam, I.
23 F., Schouten, S., Sinninghe Damsté, J. S., Talbot, H. M., and Woebken, D.: Microbial ecology
24 of the stratified water column of the Black Sea as revealed by a comprehensive biomarker
25 study, *Org. Geochem.*, 38, 2070-2097, 2007.
- 26 Wakeham, S. G., Turich, C., Schubotz, F., Podlaska, A., Xiaona, N. L., Varela, R., Astor, Y.,
27 Sáenz, J. P., Rush, D., Sinninghe Damsté, J. S., Summons, R. E., Scranton, M. I., Taylor, G.
28 T., and Hinrichs, K. U.: Biomarkers, chemistry and microbiology show chemoautotrophy in a
29 multilayer chemocline in the Cariaco Basin, *Deep Sea Res. Pt I*, 163, 133-156, 2012.

- 1 Wasmund, N., Nausch, G., and Matthäus, W.: Phytoplankton spring blooms in the southern
2 Baltic Sea - spatio temporal development and long-term trends, *J. Plankton. Res.*, 20, 1099-
3 1117, 1998.
- 4 Welander, P. V., Coleman, M., Sessions, A. L., Summons, R. E., and Newman, D. K.:
5 Identification of a methylase required for 2-methylhopanoid production and implications for
6 the interpretation of sedimentary hopanes, *PNAS*, 107, 8537-8542, 2010.
- 7 Widdel, F.: New types of acetate-oxidizing, sulfate-reducing *Desulfobacter* species, *D.*
8 *hydrogenophilus* sp. nov., *D. latus* sp. nov., and *D. curvatus* sp. nov., *Arch. Microbiol.*, 148,
9 286-291, 1987.
- 10 Xie, S., Liu, X.-L., Schubotz, F., Wakeham, S. G., and Hinrichs, K. U.: Distribution of
11 glycerol ether lipids in the oxygen minimum zone of the Eastern Tropical North Pacific
12 Ocean, *Org. Geochem.*, 71, 60-71, 2014.
- 13 Zander, J. M., Caspi, E., Pandey, G. N. and Mitra, C. R.: The presence of tetrahymanol in
14 *Oleandra wallichii*, *Phytochemistry*, 8, 2265-2267, 1969.

15
16
17
18
19
20
21
22
23
24
25
26
27
28
29
30
31
32

1 **Tables**

2

Compound	$\delta^{13}\text{C}$ [‰]		
	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
20:4 ω 6 PLFA	-30.1	-31.7	-31.6
20:5 ω 3 PLFA	-29.2	n.d.	n.d.
16:1 ω 7c 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
<i>ai</i> -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			
16:1 ω 8 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.	-

3

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

6

7

8

9

10

11

12

13

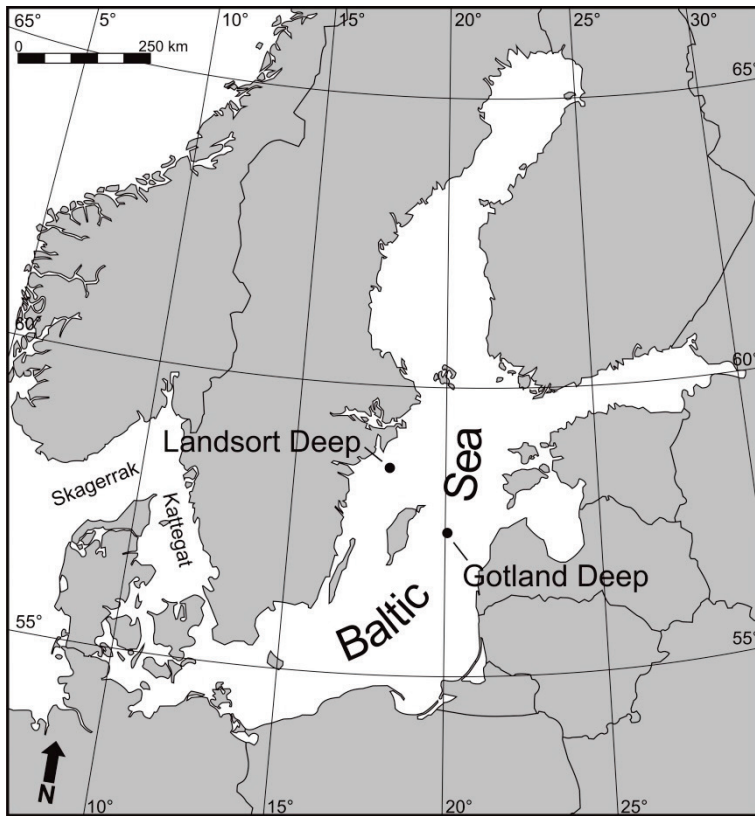
14

15

16

1 **Figures**

2



3

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

5

6

7

8

9

10

11

12

13

14

15

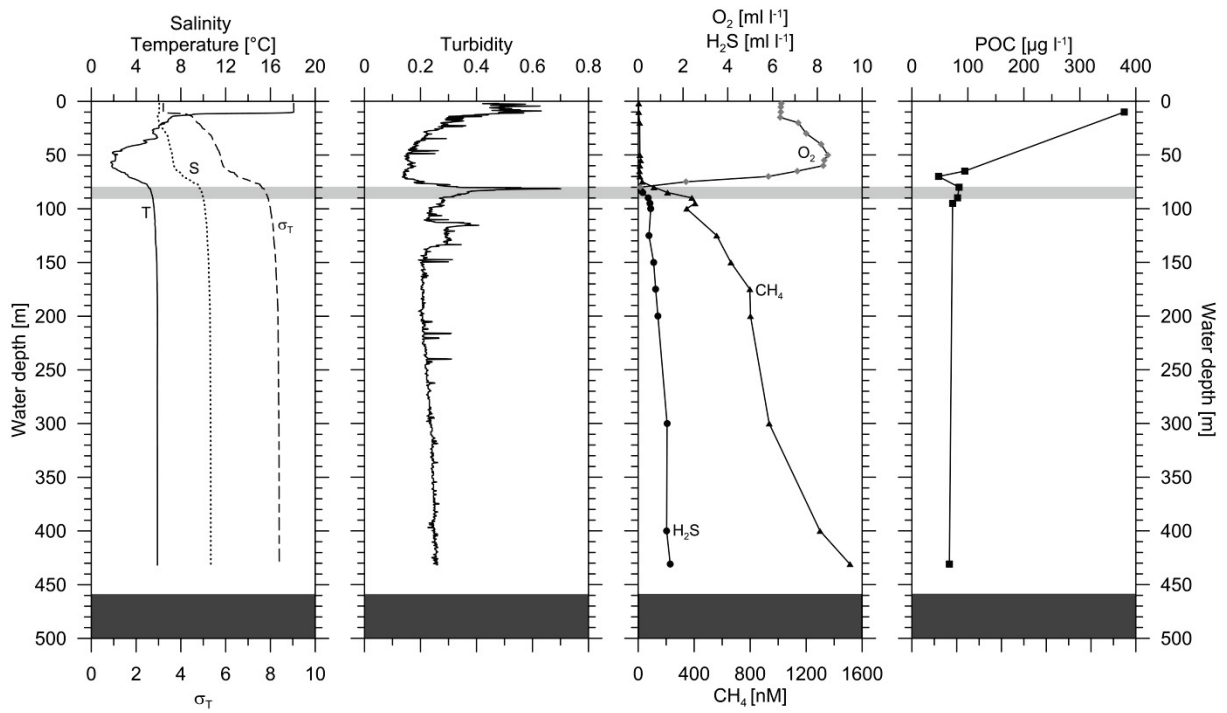
16

17

18

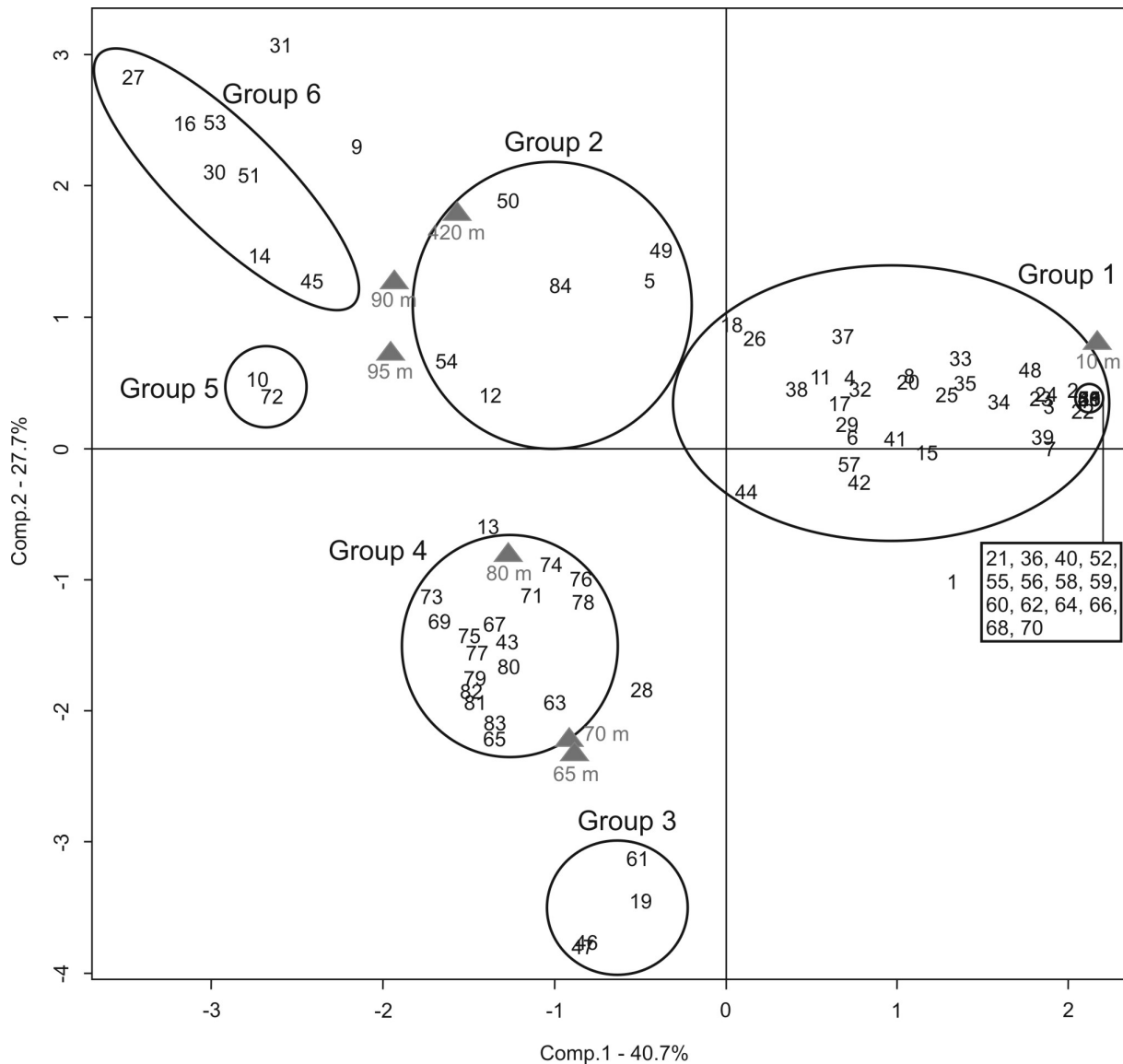
19

20



1
 2 Fig. 2: Physico-chemical characteristics of the Landsort Deep water column in summer 2011.
 3 The suboxic zone is shaded light grey. Temperature and methane data were partially taken
 4 from Jakobs et al. (2014).

5
 6
 7
 8
 9
 10
 11
 12
 13
 14
 15
 16
 17
 18
 19
 20
 21
 22



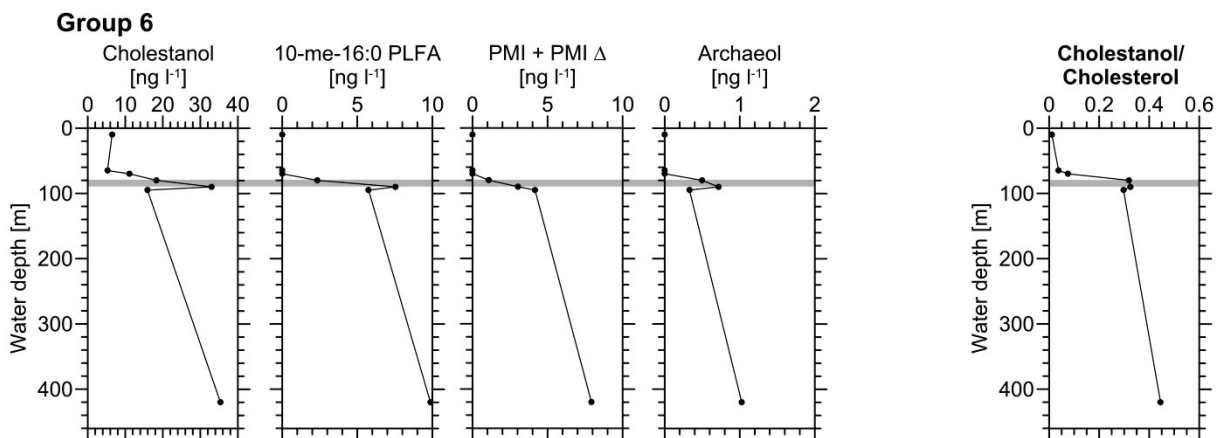
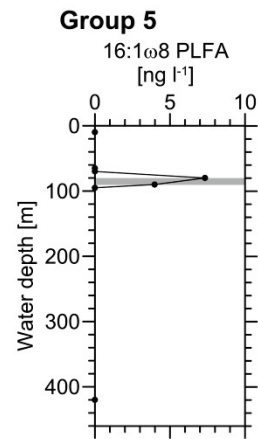
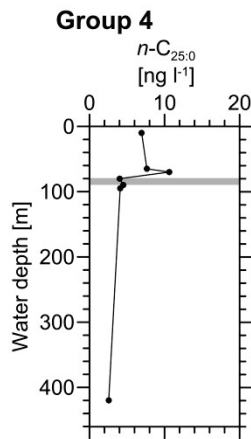
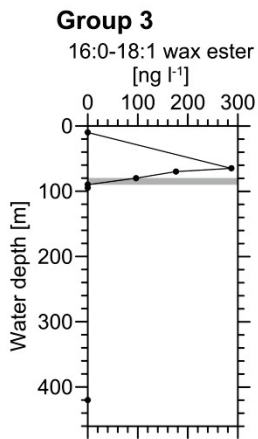
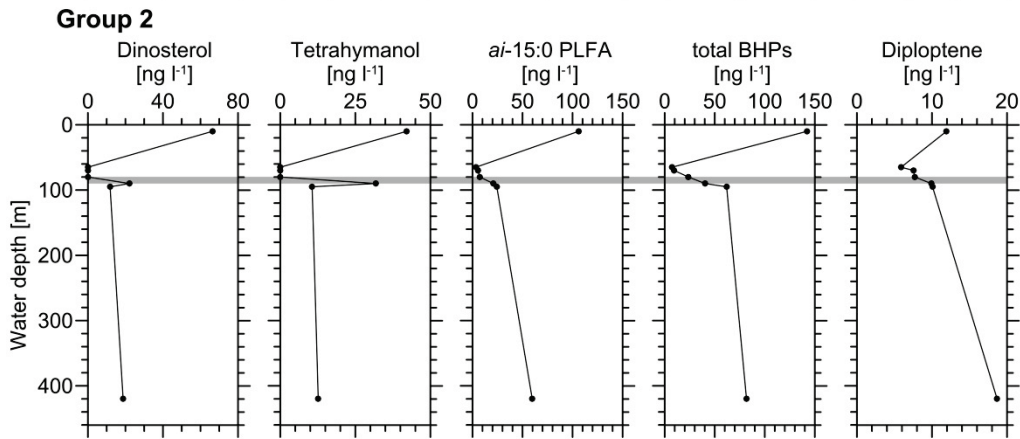
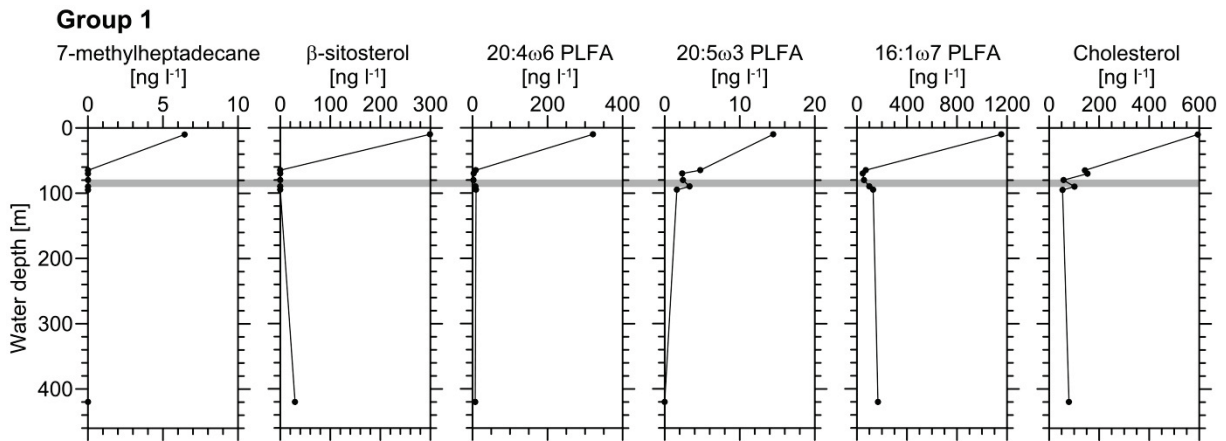
1
 2 Fig.3: PCA of the relative abundances of compounds in different water depths. Group 1:
 3 surface maximum, a subgroup of compounds exclusively occurring at the surface are listed in
 4 the box; Group 2: surface and lower suboxic zone maxima; Group 3: cold winter water layer
 5 maximum; Group 4: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

Compounds:

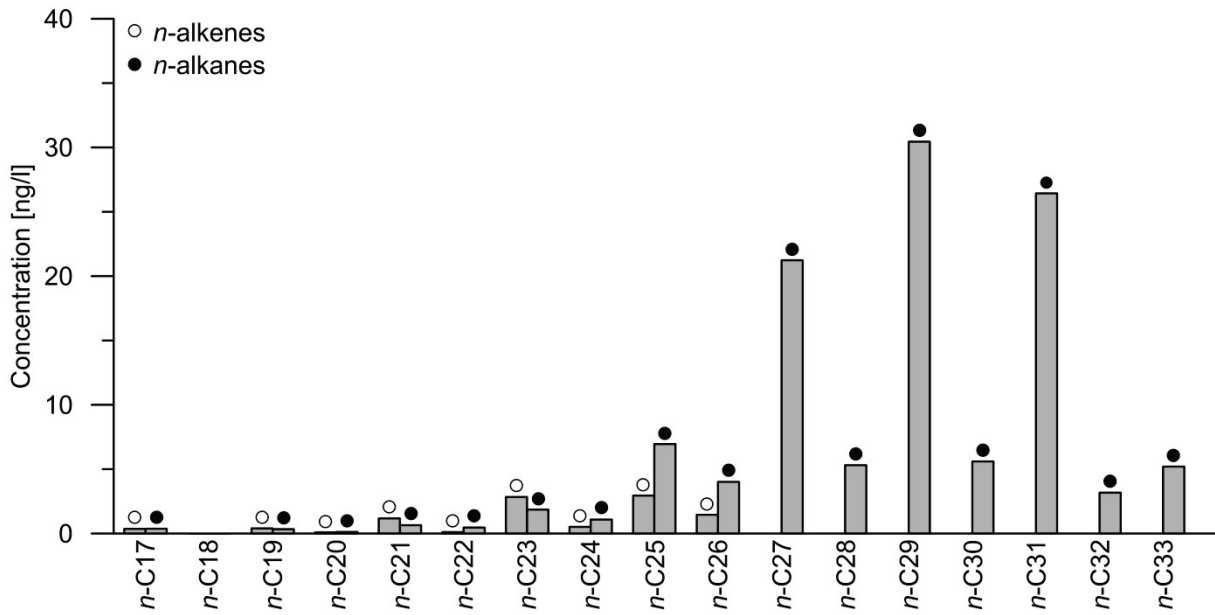
1	13:0 PLFA	22	18:4 PLFA	43	26:0 PLFA	64	<i>n</i> -C _{22:1}
2	<i>i</i> 14:0 PLFA	23	18:2 PLFA	44	cholesterol	65	<i>n</i> -C _{22:0}
3	14:0 PLFA	24	18:3 PLFA	45	cholestanol	66	<i>n</i> -C _{23:1}
4	<i>i</i> 15:0 PLFA	25	18:1 ω 9c PLFA	46	16:0-18.1 wax ester	67	<i>n</i> -C _{23:0}
5	<i>ai</i> 15:0 PLFA	26	18:1 ω 7c PLFA	47	18:0-18:1 wax ester	68	<i>n</i> -C _{24:1}

6	15:0 PLFA	27	18:1 ω 6c PLFA	48	β-Sitosterol	69	<i>n</i> -C _{24:0}
7	16:4 PLFA	28	18:1 ω 5c PLFA	49	dinosterol	70	<i>n</i> -C _{25:1}
8	<i>i</i> 16:0 PLFA	29	18:0 PLFA	50	tetrahymanol	71	<i>n</i>-C_{25:0}
9	16:1 ω 9c PLFA	30	10-me-18:0 PLFA	51	archaeol	72	<i>n</i> -C _{26:1}
10	16:1ω8c PLFA	31	<i>i</i> C19:0 PLFA	52	7-methylheptadecane	73	<i>n</i> -C _{26:0}
11	16:1ω7c PLFA	32	19:0 PLFA	53	PMI + PMI D	74	<i>n</i> -C _{27:0}
12	16:1 ω 7t PLFA	33	20:5ω3 PLFA	54	diploptene	75	<i>n</i> -C _{28:0}
13	16:1 ω 5c PLFA	34	20:4ω6 PLFA	55	<i>n</i> -C _{17:1}	76	<i>n</i> -C _{29:0}
14	16:1 ω 5t PLFA	35	20:3 PLFA	56	<i>n</i> -C _{17:0}	77	<i>n</i> -C _{30:0}
15	16:0 PLFA	36	20:3 PLFA	57	<i>n</i> -C _{18:0}	78	<i>n</i> -C _{31:0}
16	10-me-16:0 PLFA	37	20:1 PLFA	58	<i>n</i> -C _{19:1}	79	<i>n</i> -C _{32:0}
17	<i>i</i> C17:0 PLFA	38	20:0 PLFA	59	<i>n</i> -C _{19:0}	80	<i>n</i> -C _{33:0}
18	<i>ai</i> C17:0 PLFA	39	22:6 PLFA	60	<i>n</i> -C _{20:1}	81	<i>n</i> -C _{34:0}
19	17:1 PLFA	40	22:4 PLFA	61	<i>n</i> -C _{20:0}	82	<i>n</i> -C _{35:0}
20	17:0 PLFA	41	22:0 PLFA	62	<i>n</i> -C _{21:1}	83	<i>n</i> -C _{36:0}
21	18:4 PLFA	42	24:0 PLFA	63	<i>n</i> -C _{21:0}	84	total BHPs

1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18



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



3
4 Fig. 5: Concentrations of *n*-alkanes and *n*-alkenes in the Landsort Deep surface layer (oxic
5 zone, 10 m water depth).

6
7
8
9
10
11
12
13
14
15
16
17
18
19
20
21
22
23
24

1
2
3
4
5
6
7
8
9
10
11
12
13
14
15

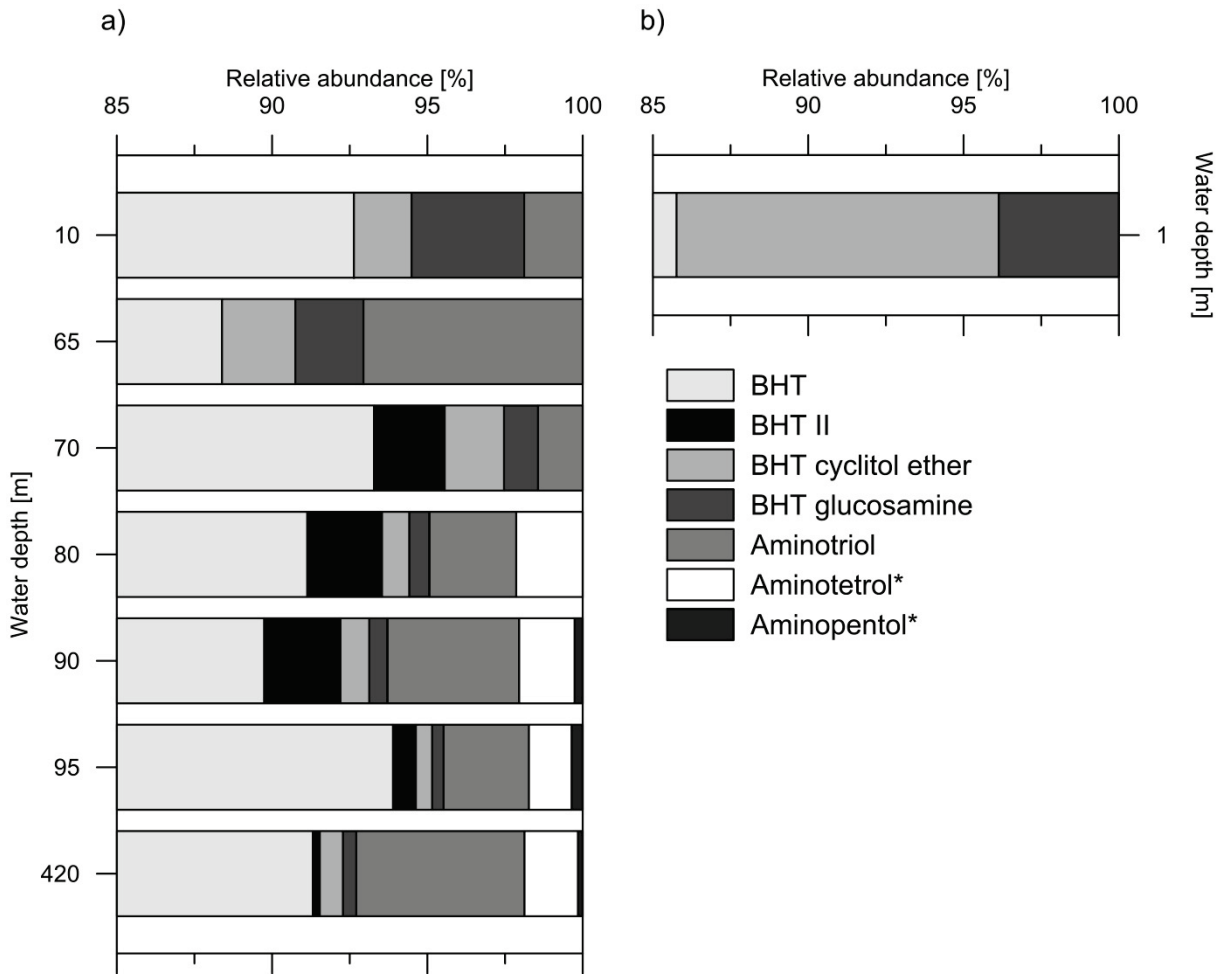


Fig. 6: Relative abundances of individual BHPs (as percent of the total) of a) the Landsort Deep water column and b) the Gotland Deep cyanobacterial bloom. Note that [%]-axes start at 85 %. * = data taken from Jakobs et al. (2014).

1

2