Particulate sulfur-containing lipids: production and cycling from the epipelagic to the abyssopelagic zone

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ABSTRACT

There are major gaps in our understanding of the distribution and role of lipids in the open ocean especially with regard to sulfur-containing lipids (S-lipids). Here, we employ a powerful analytical approach based on high resolution Fourier transform ion cyclotron resonance mass spectrometry (FT-ICR MS) to elucidate depth-related S-lipid production and molecular transformations in suspended particulate matter from the Northeast Atlantic Ocean in this depth range. We show that within the open-ocean environment S-lipids contribute up to 4.2% of the particulate organic carbon, and that up to 95% of these compounds have elemental compositions that do not match those found in the Nature Lipidomics Gateway database (termed “novel”). Among the remaining 5% of lipids that match the database, we find that sulphoquinovosyldiacylglycerol (SQDG) are efficiently removed while sinking through the mesopelagic zone. The relative abundance of other assigned lipids (sulphoquinovosylmonoacylglycerol (SQMG), sulphite and sulphate lipids, Vitamin D2 and D3 derivatives, and sphingolipids) did not change substantially with depth. The novel S-lipids, represented by hundreds of distinct elemental compositions (160 to 300 molecules at any one depth), contribute increasingly to the lipid and particulate organic matter pools with increased depth. Depth-related transformations cause (i) incomplete degradation/transformation of unsaturated S-lipids which leads to the depth-related accumulation of the refractory saturated compounds with reduced molecular weight (average 455 Da) and (ii) formation of highly unsaturated S-lipids (average abyssopelagic molecular double bond equivalents, DBE=7.8) with lower molecular weight (average 567 Da) than surface S-lipids (average 592 Da). A depth-related increase in molecular oxygen content is observed for all novel S-lipids and indicates that oxidation has a significant role in their transformation while (bio)hydrogenation possibly impacts the formation of saturated compounds. The instrumentation approach applied here represents a step change in our comprehension of marine S-lipid diversity and the potential role of these compounds in the oceanic carbon cycle. We describe a very much higher number compounds than previously reported, albeit at the level of elemental composition and fold-change quantitation with depth, rather than isomeric confirmation and absolute quantitation of individual lipids. We emphasize that saturated S-lipids have the potential to transfer carbon from the upper ocean to depth and hence are significant vectors for carbon sequestration.

*Keywords:* Sulfur-containing lipids; lipid depth-related transformations; FT-ICR MS, TLC/FID; northeast Atlantic Ocean

**1. Introduction**

Sedimentation of biogenic matter in the ocean, the so-called biological carbon pump, has a major effect on atmospheric CO2 levels. Export out of the euphotic zone takes place either through aggregation of algal cells or as faecal matter packaged by zooplankton. Lipids contribute to particulate organic matter that is directly related to the productivity of oceans. Although present at relatively low concentration in seawater, they are ubiquitous and intimately involved in numerous essential biological processes (Arts et al., 2001). Lipid molecular structure determines their reactivity and thus characterization of marine lipids on a molecular level may facilitate their use as markers for different sources of organic matter (OM) and the processes responsible for their degradation/transformation.

Molecular-level characterization of oceanic organic matter is required to address a wide range of important issues that include global climate change, surface-layer OM production and transport to the deep ocean, redistribution and fate of bioactive elements, carbon sequestration and pathways of organic matter transformation mechanisms. Despite continuous improvement in analytical methodology for OM characterization, a large fraction of oceanic OM remains uncharacterized at the molecular level, a proportion that increases with depth to include >70% of deep-ocean OM (Lee at al., 2004). The recent development of ultrahigh resolution Fourier transform ion cyclotron resonance mass spectrometry (Marshall et al., 1998) has greatly expanded the analytical window available for compositional analysis of hitherto uncharacterized marine OM.

Many aspects of lipid water column distribution and degradation in the pelagic ocean and in laboratory experiments have been investigated. The laboratory investigations on zooplanktonic lipids revealed that lipids are more labile than the bulk organic carbon in the early phase of diagenesis while the degradation rate of lipids decreased in the subsequent degradation period, demonstrating the presence of stable lipids in zooplankton, being structural lipids and waxes (Yoshimura and Hama, 2012). In a laboratory experiment, Harvey et al. (1995) have shown that lipids were more slowly degraded than proteins and carbohydrates, with oxygen having a substantial effect on overall rates of algal carbon decomposition. Following the kinetics of lipid decay of two marine phytoplankton, Harvey and Macko (1997) reported that the preserved residual lipid pool, difficult to characterize at that time, is similar to the fraction preserved in sediments. Goutx et al. (2007) have shown that lipids and other biomolecules in slower settling particles are quickly degraded while in faster settling particles the original biological signal is retained to a greater degree. Yamamoto et al. (2016) have shown that branched dialkyl glycerol tetraethers (GDGTs) are preferentially preserved in oxic environments over isoprenoid GDGTs in the NW Pacific. Investigation of certain lipid classes in the equatorial Pacific has shown polyunsaturated fatty acids are very labile and quickly lost from particles. Bacterial branched-chain C15 and C17  fatty acids increase in relative abundance as particulate matter sinks. Long-chain C37-C39 alkenones of marine origin and long-chain C20-C30 fatty acids, alcohols and hydrocarbons derived from land plants are selectively preserved in sediment (Wakeham et al., 1997). Loh et al. (2008) detected accumulations of fatty alcohols compared to other particulate organic matter components throughout the water column in the Sargasso Sea and North Central Pacific. Christodoulou et al. (2009) have shown that in the northwestern Mediterranean Sea biotic degradation of lipids generally predominates, but abiotic degradation is not negligible and, as expected, the extent of biotic degradation increases with depth.

Until recently, little data has been reported on sulfur-containing organic compounds and thus their concentrations and the processes of decomposition are largely unknown. Pohlabeln et al. (2015) and Ksionzek et al. (2016) focused their work on dissolved organic sulfur. Investigations of sulfur-containing lipids (S-lipids) were mainly related to SQDG that has been shown to play a role in the adaptation mechanism of cyanobacteria to reduce cellular phosphorus requirements when phosphorus is scarce (Van Mooy et al., 2009). Sulfolipids were investigated in algae during the middle of the 20th century (Kates and Volcani, 1966). Sulfonium analogue of phosphatidylcholine, phosphatidylsulfocholine, was discovered in non-photosynthetic (Anderson et al., 1976) as well as photosynthetic (Bisseret et al., 1984) algae. Anderson et al. (1978) characterized four major sulfolipids in non-photosynthetic marine diatom *Nitzschiu alba*, being *24-*methylene cholesterol sulfate, l-deoxyceramide-l-sulfonate, phosphatidyl sulfocholine and sulfoquinovosyl diglyceride. Only recently a new S-lipid, phosphatidyl-S,S-dimethylpropanethiol, was discovered in phytoplankton (Fulton et al., 2014).

In order to characterize sulfolipids and understand their depth-related cycling we analyzed lipid material isolated from the suspended particulate OM fraction of sub-polar Northeast Atlantic collected at the Porcupine Abyssal Plain (PAP) sustained observatory. Our analysis includes bulk lipid class quantification by thin–layer chromatography–flame ionization detection complemented by detailed compositional analysis by FT-ICR MS that provided elemental composition for 1,046 S-lipids in this sample set. FT-ICR MS has been used extensively for the characterization of oceanic dissolved organic matter (DOM)(Flerus et al., 2012). To our knowledge, this is the first report of a depth-resolved oceanic S-lipid profile from direct-infusion FT-ICR MS analysis and lipid database formula matching. Furthermore, thin–layer chromatography–flame ionization detection provided quantitative lipid characterization for specific lipid classes. We targeted lipids as relatively stable organic compounds compared to other biomolecules (Harvey et al., 1995)being important to investigate for deep ocean carbon storage.

**2. Material and methods**

*2.1. Study area, sampling, sample treatment and basic environmental data*

At the Porcupine Abyssal Plain sustained observatory (49.8N, 16.58W), in the Northeast Atlantic (Fig. 1), samples were collected at 21 depths from the surface (2 m) to 4800 m (50 m above bottom); the epipelagic (0-100 m), mesopelagic or the twilight zone (100-1000 m), bathypelagic (1000-4000 m) and abyssopelagic (4000-4850 m) zones, from the RRS James Cook on June 14, 2013, from a pre-dawn (~0400 local time) Seabird 911 + CTD-Niskin rosette. Six of the surface sampled depths (2-100 m depth) corresponded to 97, 55, 20, 7, 5 and 1% of surface irradiance intensity.



Fig. 1. PAP sampling site.

The PAP station is isolated from the complexities of the continental slope and the Mid-Atlantic Ridge. Currents are generally weak (Lampitt et al., 2001) and lateral advection speeds are low, but occasionally significant (Hartman et al., 2010). A persistent feature of the North Atlantic is the undersaturation of CO2 in surface waters throughout the year, which gives rise to a perennial CO2 sink and makes this a region of great importance in the global carbon cycle (Hartman et al., 2012).

Particulate lipids were collected on 0.7 µm Whatman GF/F filters, pre-burned at 450 °C/5 h, by filtering 5-10 l of oceanic water at 12 kPa vacuum pressures immediately after sampling. Lipids were extracted by a modified one-phase solvent mixture of dichloromethane - methanol - water procedure (1:2:0.8, v:v:v)(Bligh and Dyer,1959).

Temperature, salinity, and Chlorophyll *a* (Chl *a*) (derived from *in situ* chlorophyll-fluorescence) measurements were made using Seabird SBE 37-IM recorders (Sea-Bird Electronics Inc., Bellevue, Washington, USA). Samples for nutrient (dissolved inorganic nitrogen (DIN=nitrate + nitrite + ammonium), orthophosphate (hereinafter termed PO4) and orthosilicate (hereinafter termed SiO4)) analyses were drawn into 25 ml plastic Coulter Counter vials from Niskin bottles. The vials were stored in the dark at 4 ºC until analysis, which commenced within 24 h of sampling. Nutrients were determined in triplicate in unfiltered water samples with a Skalar Sanplus segmented flow autoanalyzer and standard colorimetric techniques described by Kirkwood (1996) and Sanders et al. (2007).

*2.2. Lipid analysis*

The particulate-derived lipid material collected was analyzed by direct-infusion electrospray ionization Fourier transform ion cyclotron resonance mass spectrometry (ESI FT-ICR MS) to provide elemental composition for sulfur-containing lipids that can serve as diagnostic markers for origin, and their transformation and preservation potential. For ESI FT-ICR MS analysis, one microgram of reserpine was added to each sample before extraction. The signal for reserpine in each final mass spectrum serves to normalize mass spectral peak magnitude for each compound to a fixed volume of seawater. FT-ICR MS data are not therefore quantitative and signal magnitudes cannot be compared for different compounds. However, signal magnitude is proportional to seawater concentration and all signal magnitudes may be monitored as an indicator of fold-change differences for a given compound between samples. Analytical standards are only available for a minute fraction of the compounds observed here, such that interpretation of ionization efficiency and mass spectral response factors (and thus quantitation) is not possible. Rather, we focus our discussion on the breadth of compositional description offered by this approach, where fold-change differences observed for each of 1,000+ S-lipids provide a means to monitor their behavior across the water column.

ESI FT-ICR mass spectrometry was performed with a hybrid linear ion trap 7 T FT-ICR mass spectrometer (LTQ FT, Thermo Fisher, San Jose, CA) equipped with an Advion Triversa Nanomate (Advion Biosystems, Inc.) as previously described (Holguin and Schaub, 2013). FT-ICR mass spectra were acquired at a mass resolving power of m/Δm50% = 400,000 at m/z 400 (i.e. a time-domain acquisition period of ∼3 s). A total of 500 time-domain transients were co-added for each sample in both positive and negative ionization modes prior to fast Fourier transformation and frequency to mass-to-charge ratio conversion. FT-ICR mass spectra were internally calibrated and peak lists were generated from each mass spectrum at S/N >10. All compounds were observed as singly charged ions, as indicated by ~1 m/z spacing between the monoisotopic and 13C112Cn-1 heavy nuclide signals. Measured masses were converted to the Kendrick mass scale (Kendrick, 1963) and sorted by the Kendrick mass defect to facilitate identification of homologous series with the same heteroatom composition and the same double-bond equivalents (DBE, the number of molecular rings plus double bonds to carbon) but differing in the degree of alkylation. High mass measurement accuracy and mass resolving power combined with Kendrick mass sorting and isotopic fine structure analysis enables unambiguous determination of elemental compositions for individual lipid compounds present in these extracts (Holguin and Schaub, 2013). Derived elemental compositions are matched to an in-house assembled lipid library derived from Lipid Maps ([http://www.lipidmaps.org/)](http://www.lipidmaps.org/). For the purposes of this report, elemental compositions for which multiple database isomeric matches are possible, further structural identification was not attempted. In cases where we discuss specific lipid molecular classes, those compounds represent elemental compositions for which only one database match is made.

Additionally, total lipid and lipid class quantitation was performed by Iatroscan thin layer chromatography/flame ionization detection (TLC/FID) (Iatroscan MK-VI, Iatron, Japan). Ten μg of hexadecanone was added to each sample before extraction for Iatroscan analysis. This internal standard was then extracted with the lipids in the sample, and the amount measured in the final concentrate provided an estimate of lipid recovery. Lipids were separated on silica-coated quartz thin-layer chromatography (TLC) rods (Chromarods SIII) (SES–Analysesysteme, Germany) and quantified by an external calibration with a standard lipid mixture. Each lipid class quantification was achieved using calibration curves obtained for representative standard by plotting peak area against the lipid amount spotted. Hydrogen flow rate was 160 mL min-1 and air flow rate was 2000 mL min-1. Each sample extract was analyzed in duplicate: for the analysis, 2 μl aliquots of 20 μl of the solution in dichloromethane were spotted by semiautomatic sample spotter. The standard deviation determined from duplicate runs accounted for 0–9% of the relative abundance of lipid classes.

The separation scheme of 18 lipid classes involve subsequent elution steps in the solvent systems of increasing polarity. For the separation of SQDG, the solvent system including solvent mixture acetone–chloroform–methanol–formic acid (33:33:33:0.6, v:v:v:v) during 40 min was used for SQDG and PG separation. Total lipid concentrations were obtained by summing all lipid classes quantified by TLC-FID and from total lipid concentration the percentage of SQDG (%SQDG) was calculated. A detailed description of the procedure is described in Gašparović et al. (2015; 2017).

*2.3. Particulate organic carbon analysis*

Seawater samples (1 l) collected from the CTD rosette were prepared by filtering onto pre-combusted 25 mm 0.7µm pore size GF/F filters and stored on board in -20 ºC for subsequent particulate organic carbon (POC) analysis. Inorganic carbonates were removed from the filters by acidification with fuming concentrated hydrochloric acid. The filters were dried in an oven at 50ºC for 24 h, packaged in pre-combusted tin capsules and analyzed with an Automated Nitrogen Carbon Analysis for Gas, Solids and Liquids (ANCA-GSL) preparation system coupled to a PDZ Europa 20-20 Stable Isotope Analyzer (PDZ Europa Scientific Instruments, Northwich, UK). The mass spectrometer can be tuned using source settings for sensitivity and/or linearity of a standard range. A typical standard range used for linearity is 25-1028 µg carbon, the limit of detection being 3 times the standard deviation of the blank of an analysis. The blank consisted of a tin capsule is analyzed in triplicate.

**3. Results and discussion**

During our sampling, temperature decreased from 13.7 °C at the surface to 2.6 °C at 4800 m (Fig. 2a). Salinity varied in the narrow range between 35.6 at the surface to 34.9 in the deep Atlantic (Fig. 2a). All nutrient concentrations increased with depth (Fig. 2b). TIN, PO4 and SiO4 concentrations ranged from 5.10 to 22.99 μmol L-1, from 0.37 to 1.89 μmol L-1 and from 0.82 to 44.35 μmol L-1, respectively. Only SiO4 was limiting for surface plankton community (Justić et al., 1995). The SiO4 depletion was probably caused by the diatom bloom. Lipid fatty acid markers also indicate a diatom bloom at our sampling site (Gašparović et al., 2016).



Fig. 2. Depth distribution of (a) temperature (T) and salinity (S) and (b) orthophosphate (PO4) (squares), orthosilicate (SiO4) (triangles) and dissolved inorganic nitrogen (DIN) (circles).

In such conditions total lipids (measured by Iatroscan TLC/FID) (Fig. 3a) peaked (24.3 µg L-1) at 30 m depth, while the lowest value (3.0 µg L-1) was measured at 3500 m depth. The total lipid concentration was lower by an average of 64% between the epipelagic and abyssopelagic zones. An even lower decrease in total lipid concentration (on average 57%) between surface and deep Eastern Atlantic Ocean was reported by Gašparović et al. (2014). S-lipids represented by SQDG standard were in the range from 0.7 µg L-1 to 3.2 µg L-1 (Fig. 3a). SQDG was the dominant lipid class observed through the entire water column. It contributed on average ~23% of the total Iatroscan-detected lipid signal with increasing contribution toward ocean depths (Fig. 3b). The contribution of SQDG to the total lipids increased considerably with depth, from an average of 16% in the epipelagic to 29% in the abyssopelagic. This observation suggests selective preservation of S-lipids throughout lipid transformation processes down the oceanic water column. SQDG is often the most abundant lipid found in different seas and oceans (Van Mooy et al., 2006; Popendorf et al., 2011a; 2011b) where SQDG (analyzed by HPLC and MS) represented roughly 30 % of the total intact polar diacylglycerolipids at many depths (Popendorf et al., 2011a). The water column POC profile shows highest POC values in the epipelagic (up to 202.3 µg L-1) and a decrease in concentration with depth (down to 24.3 µg L-1 at 4800m) (Fig. 3c). Assuming 70% carbon content in S-lipids, they contributed significantly to the POC (0.9-4.2%) (Fig. 3d) with the increasing trend toward depth. This is a remarkably high contribution when considering that S-lipids are only one class of lipids in comparison to thousands of lipid and non-lipid organic substances that contribute to the POC pool. The depth variations of total- and S-lipids follow that of Chl *a* (Fig. 3e) in the surface productive layer. This implies that photoautotrophic plankton were the main lipid producers, a feature already recognized in the Atlantic (Gašparović et al., 2014). That is not surprising knowing that the contribution of heterotrophic prokaryotes’ carbon to POC in the East Atlantic is only minor, between 1-2% (Gašparović et al., 2014) and that a high proportion of bacterial cells is not retained by the GF/F filters we used (Lee et al., 1995).



**Fig. 3.** Depth distribution of Iatroscan-determined (a) total particulate lipids (squares) and SQDG (circles), (b) the contribution of SQDG to the total lipids, (c) POC, (d) the contribution of SQDG to POC and (e) fluorescence (as a measure for Chl *a* concentration (line)) with marked depths of lipid-measured samples (horizontal lines).

The cumulative FT-ICR MS signal for total particulate lipids (Fig. 4a) demonstrated a related profile to that measured by Iatroscan (Fig. 3a), indicating cumulative signal magnitude to be an acceptably rough indicative quantitative measure of lipid content. The cumulative signal magnitude of S-lipids was lower by 81% in the abyssopelagic zone with respect to the epipelagic zone (Fig. 4b, Suppl. Fig. S1b). Box plots for the epipelagic, mesopelagic, bathypelagic and abyssopelagic zones are shown in Suppl. Fig. S1. We detected the largest decrease in lipid signal in the mesopelagic zone, which is related to the most dynamic biological and physical water column processing (Lee at al., 2004). Changes at those depths derive from the selective degradation and alteration by heterotrophic consumers that lead to the substantial alteration of the lipid composition.



**Fig. 4.** Depth-related (a) total particulate lipid (squares), S-lipid (circles) FT-ICR MS signals, (b) the number of monoisotopic S-lipid peaks (solid circles) and the contribution of number of S-lipid molecules to total number of detected lipids (open circles) and (c) the percentage of S-lipid elemental compositions that match to the Lipid Maps lipid database.



**Fig. 5.** Relationship between cumulative total lipid peak magnitude and total Iatroscan-detected particulate lipid concentrations (R=0.67, p=0.0005, n=21).

Selective preservation of S-lipids during gravitational sedimentation (*c.f*. Fig. 3b) is indicated also by the depth-related increased contribution of number of S-lipid molecules to total number of detected lipids (from an average of 21% in the epipelagic to 23% in the abyssopelagic) (Fig. 4b, Suppl. Fig. S1d). However, S-lipids show a distinct decrease in both observed number (302 to 161) (Fig. 4b, Suppl. Fig. S1c), as well as in the percentage of database-matched formulae (14 to 6%) (Fig. 4c, Suppl. Fig. S1e) with depth. Organic matter produced in the ocean euphotic zone sinks while its quantity and composition are changing. As typically <1% of the organic matter produced by phytoplankton in the surface reaches the sea floor in the open ocean (Lee at al., 2004) it is a strong argument for us to conclude that lipids in general and especially S-lipids should be the vehicle for transporting carbon to the deep ocean.

The ecological role of sulfolipids as well as parameters that influence their production within the surface layer are still poorly understood together with their depth related structural changes due to various transformation mechanisms. A step towards structural identification that illuminates S-lipid production and molecular transformation and therefore S-lipid cycling during sedimentation is the observation of depth-dependent redistribution of assigned S-lipids and those that have not been previously reported (hereafter termed “novel” S-lipids). In total, elemental compositions were assigned for 4,908 monoisotopic lipid species (i.e. when contributions from 13C and other heavy nuclides were omitted) for the entire negative ionization mode sample set. S-lipids were only observed as negative ions and a total of 1,046 monoisotopic S-lipid molecular formulae (21.3% of total lipids) were assigned across the depth profile, providing an extremely detailed view of oceanic S-lipid composition when compared to any previous report. Interestingly, of those, only 45 (4.3%) match lipid elemental compositions reported in the Nature Lipidomics Gateway (<http://www.lipidmaps.org/>) and those matching assignments include SQDG, sulphoquinovosylmonoacylglycerol (SQMG), sulphite and sulphate lipids, Vitamin D2 derivatives, Vitamin D3 derivatives and sphingolipids. In addition, those novel/unmatched S-lipids include compounds that encompass a broader range of both acyl carbon number and double bond values in comparison to S-lipid compounds previously reported. Namely, we prove that the majority of intact S-lipids, both in the surface productive layer as well as in the deep ocean, were not recognized up to now. The high proportion of unidentified S-lipids supports the premise of Hwang and Druffel (2003) that lipid-like material is a significant source of the uncharacterized organic carbon in the ocean. Furthermore, obtained oceanic S-lipid profile data seek to further illuminate the nature of OM transformation processes across the oceanic water column and inform interrogation of the oceanic total particulate lipidome.

SQDG abundance decrease massively with depth (307-fold) (Fig. 6a). Box plots for the epipelagic, mesopelagic, bathypelagic and abyssopelagic zones are shown in Suppl. Fig. S2. The differences in the abundances at various depths is compared by normalization of the total signal at each depth to the lowest cumulative signal obtained at a given depth. The highest SQDG diversity is found at epipelagic depths (18 different SQDG molecules on average), while no or only one SQDG molecular type is found below 1000 m depth (Fig. 6b, Suppl. Fig. S2b). That is not surprising since SQDG are produced predominantly by photoautotrophs (Popendorf et al., 2011b). Some SQDG found in the deepest Atlantic may indicate that other microorganisms may have the ability of SQDG synthesis. Knowing that the Niskin bottle collects a mixture of fast, slow and non-sinking particles we may also assume that some SQDG found in the deep Atlantic is effectively exported from the surface by the fast sinking mineral ballast. Fast sinking particles have been shown to be less prone to bacterial degradation than slow-sinking particles (Wakeham et al., 2009) and in addition Riley et al. (2012) concluded that fast sinking particles alone were sufficient to explain the abyssal POC flux at the PAP site.

The SQDG contribution to the overall lipid content was the highest in the euphotic zone (up to 2.9%) and dropped down to 0.1% in the abyssopelagic zone (Fig. 6c, Suppl. Fig. S2c). Moreover, we also followed the molecular weight changes (Fig. 6d, Suppl. Fig. S2d) and the degree of unsaturation (Fig. 6e, Suppl. Fig. S2e) measured by molecular double bond equivalents (DBE, the number of molecular rings plus double bonds to carbon or nitrogen for a molecule). DBE value may be corrected for heteroatoms that can form double bonds that do not contribute to aromaticity, ring formation or condensation (termed aromaticity index (AI)) (Koch and Dittmar, 2006). Thus, decrease of SQDG molecular weight between the epipelagic (average 795.9 Da) and the abyssopelagic zone (737.5 Da) parallels the decrease in degree of unsaturation. DBE values were 5.7 on average (in a range of DBE 3-9) in the epipelagic and 3.0 in the deepest Atlantic. Such relative abundance and molecular diversity depth distribution indicate that SQDG production occurs in the sunlight zone, while SQDG removal occurs at mesopelagic depths. Some SQDG residue found in the deepest Atlantic may originate from the deep-water microorganisms, possibly having different SQDG molecules with lower molecular weight and lower degree of unsaturation.

Here we also present data for other assigned S-lipids (SQMG, sulfite and sulfate lipids, Vitamin D2 derivatives, Vitamin D3 derivatives and sphingolipids) as summed abundance (Figs. 6f-j, Suppl. Fig. S2f-j). Among these, sulphite and sulphate lipid signals dominate at all depths, followed by D vitamins while sphingolipids are found only shallower than 2000 m depths. Assigned S-lipids showed a 3-fold relative abundance decrease with depth (Fig. 6f, Suppl. Fig. S2f), relatively high molecular diversity (15 different molecules at the epipelagic on average) even at the abyssopelagic (10 different molecules on average) (Fig. 6g, Suppl. Fig. S2g). Although variable, there was a relatively uniform contribution of assigned S-lipids to all lipid molecules (1.1%) across the water column (Fig. 6h, Suppl. Fig. S2h). Assigned S-lipid molecular weight was on average 461.1 Da in the epipelagic and 409.4 Da at abyssopelagic depths (Fig. 6i, Suppl. Fig. S2i). At the same time, their degree of unsaturation was higher at 4800 m depth (DBE 3.3) than at surface 2 m depth (DBE 2.6) (Fig. 6j, Suppl. Fig. S2j). This S-lipid group is composed of possibly many different S-lipid isomers. Nonetheless, they are relatively stable through the whole water column. Their increased unsaturation degree at depth may indicate adaptation of deep-water plankton to low temperature and high pressure.

We observe that the majority of assigned S-lipids, including SQDG, produced at the surface productive layer (coincident with elevated Chl *a* (Fig. 3e)), are unsaturated compounds likely produced by living plankton. They fall into two molecular mass ranges, specifically ~738-841 Da for SQDG lipids and from 452 to 472 Da for other assigned S-lipids. Furthermore, the majority (95%) of the 83 detected S-lipids that were exclusively detected within the euphotic zone (i.e. those produced and recycled within first 100 m (Table 1)), were unsaturated compounds with an average DBE value of 4.8. Moreover, 39% of those S-lipids contain nitrogen or phosphorus. This indicates the labile nature of surface produced S-lipids.

We have detected 663 novel unsaturated S-lipids with DBE ranging from 0 to 24 and molecular weight between 165 and 921 Da. The relative abundance of novel unsaturated S-lipids was 5-fold lower at bathypelagic than at epipelagic depths (Fig. 6k, Suppl. Fig. S2k). Differences also include somewhat smaller molecular diversity in the deep Atlantic (average 184 different monoisotopic elemental compositions at the surface productive layer and 127 in the abyssopelagic zone (Fig. 6l, Suppl. Fig. S2l), whose contribution to total number of observed lipid elemental compositions was 15.1% in the euphotic zone and 15.8% in the abyssopelagic (Fig. 6m, Suppl. Fig. S2m), with a correspondingly lower average molecular weight in the bathypelagic (529 Da) and abyssopelagic (572 Da) than at the epipelagic (605 Da) (Fig. 6n, Suppl. Fig. S2n) and systematic unsaturation increase from the surface productive layer (average DBE=5.0) until 4800m m depth (average DBE=8.0) (Fig. 6o, Suppl. Fig. S2o). The novel unsaturated S-lipids likely originate primarily from S-lipid transformation processes. Also, some of these compounds observed in the abyssopelagic can be partly assigned to the presence of three major plankton groups present there: bacteria, protozooplankton, and mesozooplankton (Yamaguchi et al., 2002). Abyssopelagic DOM is also rich in strongly unsaturated CHOS molecules (Hertkorn et al., 2013) which might originate from strongly unsaturated S-lipids that we found in the samples collected in the deep Atlantic. Furthermore, it was shown that heterotrophic marine bacterium *Pseudovibrio* sp. secrete metabolites that are highly unsaturated and are rich in sulfur in stressful conditions (Romano et al., 2014). Deep ocean bacteria are able to produce polyunsaturated fatty acids helping the regulation of the membrane fluidity triggered by temperature and pressure and providing protection from oxidative stress (De Carvalho and Caramujo, 2012). However, it can be assumed that the majority of novel unsaturated S-lipids are formed during their transformation processes as they sink. It is not easy to understand which processes lead to the formation of more unsaturated compounds having lower molecular mass (Figs. 6n and o, Suppl. Figs. S2n and o).

The PAP observatory site can be affected by the lateral supply of resuspended sediment, with highest resuspension in winter (Lampitt et al., 2000). Kiriakoulakis et al. (2001) noted that samples from 2 m above bottom had a very different organic composition in terms of their lipid content compared to the samples from elsewhere in the water column and suggested selective re-suspension of lipid rich organic material from the underlying sediments. Here we have found in the abyssopelagic zone an increased contribution of novel unsaturated S-lipids having increased molecular weight when compared to the bathypelagic. We assume that novel secondary produced unsaturated S-lipids at abyssopelagic depths can be generated *in situ* via "quenching" of functionalized lipids by sulfur at the early stages of sediment diagenesis and even in the water column (Sinninghe Damsté, and de Leeuw, 1990). The "sulfur quenching" process acts as a trapping mechanism of labile functionalized lipids.

The relative abundance of saturated S-lipids (DBE=0) was moderately (55%) lower in the abyssopelagic zone than at the surface (Fig. 6p, Suppl. Fig. S2p). The number of different saturated S-lipid formulae increased from the ocean surface to the bottom layer (37 to 49) (Fig. 6q, Suppl. Fig. S2q). The saturated S-lipids are primarily non-aromatic in their molecular structure as illustrated from the AI index (Koch and Dittmar, 2006) of S-lipids across the water column, a value which was zero for most observed S-lipids. Only at bathypelagic and abyssopelagic depths, were there a few S-lipid formulae found to have AI values greater than 0.5, implying their aromatic structure. The contribution of saturated S-lipid molecules to all lipid molecules increased from 1.9% at the surface (2 m depth) to 8.5% at 4800 m depth (Fig. 6r, Suppl. Fig. S2r). Their increased molecular diversity with depth together with their depth related increased molecular contribution among all lipid molecules point to their depth related formation and selective preservation. The average molecular mass of the saturated S-lipids varied slightly around the value of ~455 Da through the entire water column (Fig. 6s, Suppl. Fig. S2s). For comparison, average molecular mass of saturated novel nitrogen-containing lipids increased from an average of 605 Da at the epipelagic to 713 Da at abyssopelagic depths (Gašparović et al., 2017).



**Fig. 6.** Depth-related (a, f, k, p) relative abundance, (b, g, l, q) the number of compounds, (c, h, m, r) the contribution of compound number to the total number of detected lipids, (d, i, n, s) average molecular mass and (e, j, o) average double bond equivalents of (a-e) sulphoquinovosyldiacylglycerol (SQDG), (f-j) other assigned S-lipids, (k-o) novel unsaturated and (p-s) novel saturated S-lipids obtained in negative-ion ESI FT-ICR MS.

Depth-related molecular transformation of lipids include biotic (enzymatic peroxidation, biohydrogenation (Rontani and Koblížek, 2008)) and abiotic (photooxidation and autoxidation) degradation (Rontani et al., 2011, and references therein). With the objective of proposing a transformation mechanisms for S-lipids, in Fig. 7 we present the depth-related average hydrogen to carbon (H/C) and oxygen to carbon (O/C) ratios of novel saturated and unsaturated S-lipids that appeared to be the major S-lipid fraction at the Northeast Atlantic PAP station. H/C ratios of saturated S-lipids were uniform throughout the water column, while the O/C ratio increased with depth. In the case of the unsaturated S-lipids, H/C ratio decreased and at the same time O/C ratio increased with depth. Both H/C and O/C ratios were substantially higher for the saturated (average 2.06 and 0.34, respectively) than those for the unsaturated S-lipids (average 1.67 and 0.21, respectively). Depth-related increased oxygen content in the S-lipids indicates that oxidation is an important mechanism for their depth-related transformation. The highest average H/C ratio of novel saturated S-lipids indicate that biohydrogenation is probably also an important mechanism for their formation. Marine bacteria and fungi were shown to perform biohydrogenation (Rhead et al., 1971; Wakeham, 1989; Ferreira et al., 2015).



Fig. 7. Depth-related (a) average hydrogen to carbon (H/C) and (b) oxygen to carbon (O/C) ratios of novel S-lipids which were unsaturated (diamonds) or saturated (squares).

Additionally, we monitor those S-lipids that can be traced from the surface productive layer to the deep ocean and as such are potentially important as deep ocean carbon storage compounds since OM from the particles below the euphotic zone is no longer able to contribute to the biogeochemistry of the upper ocean (Lampitt et al., 2010). We observed 40 S-lipid compounds that are continuously present in the water column from the surface to the deep Atlantic (Table 2). The relative abundance value shows the depth-dependent distribution as illustrated for three S-lipids continuously present in the water column and shown in Fig. 8. The majority of these S-lipids have a DBE value of zero suggesting that saturated S-lipids have the potential for transferring carbon from the surface to the deep ocean and therefore may contribute considerably to carbon sequestration.



**Fig. 8.** Depth distributions of the relative concentrations of (a) SQDG compound(s) C37H69O12S1, (b) C24H49O9S1 and (c) C16 H33O4S1. Relative concentration is calculated by normalization of the peak magnitude at each depth to the lowest measured peak magnitude across the depth profile.

The chemical identity of S-lipids in the deep ocean is necessary to resolve in order to understand their transformation mechanisms as well as their contribution to the pool of fossil organic matter, linking these compounds to the processes of carbon sequestration. Our data shed preliminary understanding on this question and indicate that 35% of S-lipids found at the bottom (4800 m depth) are saturated compounds (Fig. 9). This confirms a substantial contribution of saturated S-lipids in the deep Atlantic and implicates them in deep carbon burial/sequestration.



Fig. 9. Number of saturated (DBE=0) and unsaturated (DBE>0) novel S-lipids found at 4800 m depth.

**4. Summary and conclusions**

The abundance of sulfur lipids, their ecological role and the parameters that influence their production in the surface layer are poorly understood at present, together with their depth-related structural changes due to various transformation mechanisms. Our data are the first step in resolving these unknowns.

In the Northeast Atlantic Ocean we have identified 1,046 sulfolipid molecular formulae (monoisotopic) among which only 45 (4.3%) match the lipid elemental compositions previously reported in the Nature Lipidomics Gateway, indicating the magnitude of a gap in scientific knowledge on S-lipids as an integral part of uncharacterized oceanic organic matter.

Here we show that SQDG are efficiently removed from the water column within the mesopelagic zone. A proof of selective preservation of novel S-lipids through lipid transformation processes across the oceanic water column highlight their potential to contribute significantly to global ocean inventory budget. Based on the continuous decrease in both the number and abundance of unsaturated novel S-lipids and the decrease in their average molecular mass with depth we conclude that S-lipid cycling and transformation during sedimentation includes the incomplete degradation of unsaturated S-lipids that leads to the accumulation of degradation-resistant saturated compounds with reduced molecular weight (on average 455 Da). This would be in addition to remineralization to CO2, and/or dissolution. Saturated S-lipids may have the potential to transfer carbon from the upper ocean to depth and hence they are significant vectors for carbon sequestration. Furthermore, here we show that S-lipids are involved in the transformation processes that lead to the formation of novel lipids of very high unsaturation in the deep Atlantic.

Depth-related increased oxygen content in all novel S-lipids indicate that oxidation has a significant role in their transformation processes. However, (bio)hydrogenation should have a role in the formation of saturated compounds. There is also a high probability that some novel secondary produced unsaturated S-lipids at abyssopelagic depths can be in situ generated via "quenching" of functionalized lipids by sulfur.

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**Table 1.** Assigned elemental composition, nominal molecular mass (Mw), tentative molecular identification (derived from Lipid Maps, Nature Lipidomics Gateway) and corresponding double bond equivalents (DBE) of S-lipid mass spectral signals exclusively found at the surface productive layer.

|  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- |
| Elemental Composition | Mw | Molecular identification | DBE | Elemental Composition | Mw | Molecular identification | DBE |
| (Da) | (Da) |
| C33H64O11S1 | 668.4175 |  | 2 | C17H35O1S1P1 | 318.2152 |  | 1 |
| C35H68O11S1 | 696.4488 |  | 2 | C19H39O3S1P1 | 378.2363 |  | 1 |
| C36H71N1O7S1 | 661.4957 |  | 2 | C19H37O3S1P1 | 376.2207 |  | 2 |
| C37H72O11S1 | 724.4801 |  | 2 | C19H36O2S1 | 328.2442 |  | 2 |
| C37H70O11S1 | 722.4644 |  | 3 | C22H45O12S1P1 | 564.2375 |  | 1 |
| C37H67N1O7S1 | 669.4644 |  | 5 | C23H46O5S1 | 434.3072 |  | 1 |
| C38H74O11S1 | 738.4957 |  | 2 | C24H50O5S1 | 450.3384 |  | 0 |
| C39H77N1O13S1 | 799.5121 |  | 2 | C24H48O4S1 | 432.3279 |  | 1 |
| C39H73N1O6S1 | 683.5164 |  | 4 | C26H54O5S1 | 478.3698 |  | 0 |
| C39H70O12S1 | 762.4594 |  | 5 | C26H52O4S1 | 460.3592 |  | 1 |
| C40H78O5S1 | 670.5576 |  | 2 | C26H50O8S1 | 522.3232 |  | 2 |
| C40H70O10S1 | 742.4695 |  | 6 | C26H48O8S1 | 520.3075 |  | 3 |
| C41H81N1O13S1 | 827.5434 |  | 2 | C29H58O6S1 | 534.396 |  | 1 |
| C41H80O11S1 | 780.5427 |  | 2 | C29H59N1O5S1 | 533.4119 |  | 1 |
| C41H78O11S1 | 778.527 |  | 3 | C30H61N1O6S1 | 565.4183 |  | 1 |
| C41H74O12S1 | 790.4907 | SQDG | 5 | C31H65O2S1P1 | 532.4448 |  | 0 |
| C41H72O12S1 | 788.475 |  | 6 | C31H63O2S1P1 | 530.4292 |  | 1 |
| C41H70O12S1 | 786.4594 |  | 7 | C31H58O7S1 | 574.3909 |  | 3 |
| C41H68O12S1 | 784.4437 |  | 8 | C32H65N1O7S1 | 607.4487 |  | 1 |
| C42H74O4S1 | 674.5313 |  | 6 | C32H65N1O6S1 | 593.4496 |  | 1 |
| C42H73N1O6S1 | 719.5164 |  | 7 | C32H62O4S1 | 542.4374 | Sulfate Lipid | 2 |
| C42H71N1O6S1 | 717.5008 |  | 8 | C32H60O4S1 | 540.4218 |  | 3 |
| C43H82O11S1 | 806.5583 |  | 3 | C32H59N1O5S1 | 569.4119 |  | 4 |
| C43H76O12S1 | 816.5063 | SQDG | 6 | C33H68O7S1 | 608.4691 |  | 0 |
| C43H74O12S1 | 814.4907 |  | 7 | C33H67O2S1P1 | 558.4605 |  | 1 |
| C43H72O12S1 | 812.475 |  | 8 | C33H62O7S1 | 602.4222 |  | 3 |
| C44H87O12S1P1 | 870.5661 |  | 2 | C34H68O5S1 | 588.4793 |  | 1 |
| C44H78O5S1 | 718.5576 |  | 6 | C34H66O5S1 | 586.4637 |  | 2 |
| C44H75N1O6S1 | 745.5321 |  | 8 | C34H63N1O7S1 | 629.4331 |  | 4 |
| C45H74O12S1 | 838.4907 | SQDG | 9 | C34H61N1O7S1 | 627.4174 |  | 5 |
| C45H74O11S1 | 822.4957 |  | 9 | C34H60O7S1 | 612.4065 |  | 5 |
| C45H72O12S1 | 836.475 | SQDG | 10 | C35H69N1O7S1 | 647.4800 |  | 2 |
| C45H70O12S1 | 834.4594 | SQDG | 11 | C35H63N1O7S1 | 641.4331 |  | 5 |
| C46H82O5S1 | 746.5889 |  | 6 | C36H65N1O7S1 | 655.4487 |  | 5 |
| C50H99N1O7S1 | 857.7148 |  | 2 | C36H62O2S1 | 558.4476 |  | 6 |
| C50H97N1O7S1 | 855.6991 |  | 3 | C36H60O2S1 | 556.432 |  | 7 |
| C51H68O2S1 | 744.4946 |  | 18 | C40H70O4S1 | 646.5 |  | 6 |
| C52H103N1O7S1 | 885.7461 |  | 2 | C40H67N1O4S1 | 657.4796 |  | 8 |
| C52H101N1O7S1 | 883.7304 |  | 3 | C42H56O1S1 | 608.4057 |  | 15 |
| C53H66O2S1 | 766.4789 |  | 21 | C44H60O1S1 | 636.437 |  | 15 |
| C55H70O2S1 | 794.5102 |  | 21 |   |  |  |  |

 1sulphoquinovosyldiacylglycerol

 **Table 2**. Assigned elemental composition, nominal molecular mass (Mw), tentative molecular identification (derived from Lipid Maps, Nature Lipidomics Gateway), double bond equivalents (DBE) and decrease of relative abundance between the surface productive and bottom layer (4800 m) for the 40 S-lipid mass spectral signals which trace may be followed from the surface until the depth.

|  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| Elemental Composition | Mw(Da) | Molecular identification | DBE | Abundancedepth decrease(fold-change) | Elemental Composition | Mw(Da) | Molecular identification | DBE | Abundancedepth decrease(fold-change) |
| C12H26O4S1 | 266.15573 |  | 0 | 1.3 | C22H46O9S1 | 486.28681 |  | 0 | 1.8 |
| C14H30O4S1 | 294.18702 |  | 0 | 1.2 | C24H50O9S1 | 514.31811 |  | 0 | 2.6 |
| C14H30O5S1 | 310.18194 | Sulfate Lipid | 0 | 1.3 | C24H50O10S1 | 530.31302 |  | 0 | 2.1 |
| C16H26O3S1 | 298.16081 |  | 4 | 1.0 | C26H54O10S1 | 558.34432 |  | 0 | 3.1 |
| C16H34O4S1 | 322.21832 |  | 0 | 1.2 | C26H54O11S1 | 574.33923 |  | 0 | 2.0 |
| C16H34O5S1 | 338.21324 | Sulfate Lipid | 0 | 1.5 | C27H34O2S1 | 422.22850 |  | 11 | 3.8 |
| C16H34O6S1 | 354.20815 |  | 0 | 1.4 | C27H46O4S1 | 466.31223 | Sulfates  | 5 | 9.3 |
| C17H28O3S1 | 312.17646 | Sulfite Lipid | 4 | 1.1 | C28H46O4S1 | 478.31223 | Sulfates  | 6 | 20.4 |
| C18H30O3S1 | 326.19211 | Sulfite Lipid | 4 | 1.1 | C28H58O11S1 | 602.37053 |  | 0 | 2.8 |
| C18H38O4S1 | 350.24962 |  | 0 | 8.3 | C28H58O12S1 | 618.36545 |  | 0 | 2.1 |
| C18H38O5S1 | 366.24454 |  | 0 | 4.8 | C29H50O4S1 | 494.34353 |  | 5 | 10.9 |
| C18H38O6S1 | 382.23945 | Suflate Lipid | 0 | 1.8 | C30H62O12S1 | 646.39675 |  | 0 | 3.3 |
| C18H38O7S1 | 398.23437 |  | 0 | 1.6 | C30H62O13S1 | 662.39167 |  | 0 | 2.3 |
| C19H32O3S1 | 340.20776 |  | 4 | 1.1 | C32H60O8S1 | 604.40144 |  | 3 | 2.6 |
| C20H38O7S1 | 422.23437 |  | 2 | 5.3 | C32H62O6S1 | 574.42726 |  | 2 | 11.9 |
| C20H40O5S1 | 392.26019 |  | 1 | 1.3 | C32H64O6S1 | 576.44291 |  | 1 | 21.9 |
| C20H42O6S1 | 410.27076 |  | 0 | 2.1 | C32H65N1O6S1 | 591.45380 |  | 1 | 79.4 |
| C20H42O7S1 | 426.26568 |  | 0 | 2.3 | C32H66O14S1 | 706.41788 |  | 0 | 4.0 |
| C20H42O8S1 | 442.26059 |  | 0 | 1.8 | C37H70O11S1 | 738.45935 | SQDG | 3 | 56.1 |
| C22H46O8S1 | 470.29189 |  | 0 | 2.4 | C60H77O2S1P1 | 892.53873 |  | 23 | 2.4 |

1sulphoquinovosyldiacylglycerol