Free fatty acids, tri-, di- and monoacylglycerol production and depth-related cycling in the Northeast Atlantic

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**Abstract**

We present the characterisation and vertical distribution of suspended particulate lipids containing C, H and O which have the potential to sequester carbon from the upper ocean when associated with sinking particles. Lipids have been shown to be valuable in a host of environments to provide insights into the sources and processing of organic materials in the oceans. Here we present, direct-infusion, high resolution Fourier transform ion cyclotron resonance mass spectrometry (FT-ICR MS) combined with bulk lipid measures for marine lipid characteriazation. We present the water column distribution of free fatty acids, tri-, di- and monoacylglycerols from the surface layer to abyssopelagic depths (4800 m) for samples collected in the Northeast Atlantic at the Porcupine Abyssal Plain sustained observatory (PAP-SO) (49.0 °N, 16.5 °W). Triacylglycerols (TG) with even carbon number (TG) and odd carbon number (oddTG, reflecting bacterial origin), were analysed, while free fatty acids were analysed as unsaturated (UFA), branched (BrFA) and saturated (SAFA) fatty acids. The surface productive layer (euphotic zone) was characterized with the highest incidence of lipids that are not reported in the Nature Lipidomics Gateway database, especially lipids that are highly unsaturated (acyl chain unsaturation was on average 3.8 for TG, oddTG, UFA and diacylglycerols (DG)). Additionally, we observed high lipid degradation at epipelagic depths. Fatty acid markers indicate that diatoms and dinoflagellates were important contributors to the lipid pool. Depth-resolved lipid change includes decreased lipid abundance and molecular diversity together with substantial loss of unsaturation with increasing depth. The major lipid change occurs at upper mesopelagic depths. Unlike other observed lipids, the abundance of SAFA remained essentially constant down the water column whereas the number of SAFAs and their contribution to total lipids increased with depth. Thus, we demonstrate that lipid saturation affects the export of carbon from the atmosphere to the deep ocean.

Keywords: lipid production, lipid cycling, Northeast Atlantic, FT-ICR MS

1. **Introduction**

The cycling of organic carbon in the marine environment is a key process in the global carbon cycle. A major source of organic carbon in the oceans is autotrophic production by phytoplankton (Falkowski et al., 1998). Although autotrophic production occurs in the surface productive euphotic zone (0-200 m depth), gravitational sinking provides the major pathway for transportation of particulate organic carbon from the surface layer to the ocean depths. During sinking, organic matter (OM) is selectively transformed with a spectrum of turnover rates and fluctuation in OM concentration depends on OM composition.

Autotrophic plankton is the main lipid source in the oceans (Gašparović et al., 2014). The lipid content of phytoplankton cells ranges from ≤1% to 46% of dry weight (Romankevich, 1984). Particulate organic matter (POM) in the Ross Sea has lipid, protein and carbohydrate contributions to particulate organic carbon (POC) in the photic layer of 7.4, 33.2 and 20%, respectively. Below the photic layer the lipid/protein/carbohydrate proportions are 10.6, 18.5 and 26% (Fabiano et al., 1993). The average composition of lipids, carbohydrates, and proteins in the surface northern Chukchi Sea were 50, 35 and 15% for POM, respectively (Kim et al., 2015). The average contribution of particulate lipids to POC in the Northern Adriatic ranges from 14 to 38% (Frka et al., 2011; Marić et al., 2013).

Lipids are carbon rich, with high energy content, and represent important metabolic fuels. They differ to a substantial degree in chemical structure and functionality. There is a variety of different lipids in the marine water column attesting to the diversity of biosynthetic pathways employed by aquatic organisms. Wax esters (WE) are major neutral lipids in some zooplankton species (Kattner, 1989) and in their detritus and faecal pellets (Wakeham et al., 1984). Fatty alcohols (ALC) mainly originate from zooplankton wax esters. Polar lipids, i.e. phospholipids (phosphatidylglycerols, phosphatidylethanolamines, phosphatidylcholines), and glycolipids (sulfoquinovosyldiacylglycerols, monogalactosyldiacylglycerols, digalactosyldiacylglycerols) are biomembrane structure components and reveal the organic matter associated with living organisms (Derieux et al., 1998). Triacylglycerols (TG) are storage lipids in many phytoplankton species. The fatty acid amount and composition is dependent on both the growth conditions and the physiological state (Volkman, 2006). The level of TG accumulation is variable, from ~2% to 77% and may be stimulated by a number of environmental factors. Nitrogen deprivation has a major impact on TG synthesis, and many algae show a two to three-fold increase in lipid content, predominantly TG, under nitrogen limitation (Thompson, 1996). Algal TG are generally characterized by saturated and monounsaturated fatty acids. However, some species have demonstrated a capacity to accumulate high levels of long chain polyunsaturated fatty acids in TG (Guschina and Harwood, 2009). Diacylacylglycerols (DG), monoacylacylglycerols (MG) and free fatty acids (FFA) are acylglycerol breakdown products and characterize degradation level (Parrish, 1988; Goutx et al., 2003). The lipolysis index (LI) can be used as a measure of lipid degradation in sea water (Goutx et al., 2003). Different lipid molecular structures influence reactivity and thus preservation potential. However, molecular structure is not the only factor that affects t OM reactivity, as it also depends on environmental conditions (Wakeham and Canuel, 2006).

Lipid cycling in the equatorial Pacific has shown that fatty acids are labile compounds, with polyunsaturated fatty acids being 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 areselectively 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. However, organic chemical compositions of sinking particles vary as a function of *in situ* particle settling velocity (Wakeham et al., 2009). The spatial and temporal variation in the organic composition of suspended particles in the equatorial Paciﬁc Ocean have shown that surface suspended particles (0–200 m) are similar in composition to surface ocean phytoplankton and are less degraded than particles sinking out of the euphotic zone (105m). Midwater suspended particles (200–1000 m) contain labile phytodetrital material derived from particles exiting the euphotic zone (105 m) (Sheridan et al., 2002).

In a laboratory experiment, Harvey et al. (1995) have shown large differences in decay rates among major biochemical classes (proteins, carbohydrates, and lipids), with carbohydrates utilized most rapidly, followed by proteins and then lipids. Turnover rate among particulate OM pools ranged from 10 days for carbohydrates under oxic conditions to over 160 days for lipids under anoxia, with oxygen having a substantial effect on overall rates of algal carbon decomposition. A general increase with depth in the percentage of POC contributed by lipids was found in the Ross Sea indicating their selective preservation potential (Fabiano et al., 1993). This indicates that lipids are an important biochemical group in the processing of OM. In contrast, Wakeham et al. (1997) found in the equatorial Pacific that lipids were, in general, selectively lost due to their greater reactivity relative to bulk organic matter toward biogeochemical degradation in the water column. Such difference in lipid diagenetic reactivity might be ascribed to the different composition of lipids that are produced at the surface layer with diverse levels of reactivity (Wakeham et al., 1997).

There is a need to understand ocean carbon cycles and the role of organic matter in the oceans particularly with regard to their capacity to sequester carbon from the atmosphere. A crucial process in this is the generation of carbon-rich sinking material in the upper ocean. Despite interest and continuous improvement of analytical approaches for molecular-level OM characterization, a large fraction of oceanic OM is still not characterized; the proportion of the uncharacterized fraction increases with depth, contributing 70-80% in the deep ocean (Lee et al., 2004). We employ uniquely powerful mass spectrometry, FT-ICR MS, which provides both high mass resolving power and sub-part-per-million mass measurement accuracy (a feature that allows elemental composition to be determined directly and unambiguously from measured mass to charge ratio for these analyses). With this approach, we identify thousands of lipid compounds present in each lipid extract to provide a comprehensive qualitative description of the lipidome at the level of elemental composition. We apply database matching/sorting for identified elemental compositions to provide putative elemental composition to lipid molecule where possible using the Nature Lipidomics Gateway database of known lipids. We evaluate the role of triacylglycerols and lipid degradation products such as DG, MG and FFA in exporting carbon to the deep ocean. Given that those lipids do not possess the elements nitrogen and phosphorus, we expected them to be stable and therefore important vectors for carbon sequestration on a global scale. To address that, we analyzed the depth-dependent lipid profiles for particulate lipid extracts from the sub-polar Northeast Atlantic collected at the Porcupine Abyssal Plain sustained observatory (PAP-SO) (49.0 °N, 16.5 °W). We employ FT-ICR MS and thin–layer chromatography–flame ionization detection to provide qualitative and quantitative lipid characterization and to monitor lipid molecular change for sinking lipids.

**2. Material and Methods**

Sampling was carried out on June, 14th 2013 at the Porcupine Abyssal Plain sustained observatory (49.0 °N, 16.58 °W) in the subpolar Northeast Atlantic. The PAP-SO station is isolated from the complexities of the continental slope and the Mid-Atlantic Ridge and thus can be considered as a representative of a large area of the open ocean (Lampitt et al., 2010). A persistent feature of the North Atlantic is the undersaturation of CO2 in surface waters throughout the year, giving rise to a perennial CO2 sink which makes it a region of great importance in the global carbon cycle (Hartman et al., 2012) and an important site to investigate carbon sinking and mechanisms of its transformation. Samples were collected by Niskin samplers at 21 depths from surface (2 m) to 4800 m (50 m above bottom) in June 2013.

Particulate lipids were collected on 0.7 µm Whatman GF/F filters (pre-combusted for 5 h at 450°C)by filtering 5-10 l of oceanic water at 12 kPa vacuum pressures immediately after sampling and stored at -80°C. Ten micrograms of internal standards n-hexadecanone (for Iatroscan analysis) and 1 µg reserpine (for FT-ICR MS analysis) were added to each sample before extraction. The final measured amount provided lipid recovery estimation. 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). The extracts were evaporated to dryness under nitrogen atmosphere and stored at -20°C until analysis.

The particulate-derived lipid material collected from this sampling was analyzed by direct-infusion electrospray ionization Fourier transform ion cyclotron resonance mass spectrometry (ESI FT-ICR MS) to provide elemental composition determination for lipid compounds that can serve as diagnostic markers for origin, their transformation and preservation potential through 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). High mass measurement accuracy and mass resolving power combined with Kendrick mass sorting and isotopic fine structure analysis provided an unambiguous analysis of the elemental composition for individual lipid compounds present in these extracts. Assigned elemental compositions were matched to a lipid library derived from Lipid Maps ([http://www.lipidmaps.org/)](http://www.lipidmaps.org/) for tentative lipid molecular assignment.

Additionally, total lipid and lipid class quantitation was performed by IATROSCAN thin layer chromatography/flame ionization detection (TLC/FID) (Iatroscan MK-VI, Iatron, Japan). Lipids were separated on Chromarods-SIII and quantified by an external calibration with a standard lipid mixture. Hydrogen flow was 160 ml/min and air flow was 2000 ml/min. Detailed procedures are described in Gašparović et al. (2015). The lipolysis index (LI) was calculated as the ratio of the sum of lipid metabolites (ALC+FFA+MG+DG) to the sum of TG, WE, and glyco- and phospholipids (Goutx et al., 2003). Temperature, salinity, and chlorophyll *a* (Chl *a*) measurements were made using Seabird SBE 37-IM recorders (Sea-Bird Electronics Inc., Bellevue, Washington, USA).

**3. Results**

The PAP station environmental conditions were reflected in temperature that varied between 13.7°C at the surface and 2.6°C at 4800 m (Fig. 1a), while salinity varied in the range of 35.6 at the surface to 34.9 at 4800m (Fig. 1b). Chl *a*, measured by chlorophyll fluorescence (Fig. 1c), was higher from the surface to 50 m depth, with the highest value observed at 30 m.

Suspended total lipids, TG, DG, MG, FFA (Fig. 2a, Appendix Table 1) were higher in the surface productive euphotic zone following Chl *a* distribution (Fig. 1c). The total lipid concentrations ranged from 3.0 to 24.9 μg L-1, with maxima observed at 30 m depth as observed for all the lipid classes discussed here. The TG, DG, MG and FFA concentrations were as high as 1.9, 0.39, 0.1, 3.5 μg L-1 at 30 m, respectively and then decreased below 0.1 μg L-1 at abyssopelagic (≥ 4000 m) depths.

The observed lipid class contributions to total lipids in the water column decreased in the order of FFA>TG>DG>MG (Fig. 2b). Free fatty acids contributed between 0.8% at 1500 m depth and 15.7% at 5 m depth, DG contributed between 0.4% (1500 m depth) to 2.9% (2000 m depth) and MG between contributed 0.1% (100 m depth) to 0.7% (3000 m depth). The highest contribution of TG to the total lipid signal (i.e. 7.9%) was observed in the epipelagic zone (0-200 m), whereas TG contribution decreased to 3.2% from mesopelagic to abyssopelagic zone.

The water column POC profile shows surface POC enrichment (up to 202.3 µg L-1) and a decrease in concentration with depth (down to 24.3 µg L-1) (Fig. 2c). We do not have POC data for the whole water column due to the irreparable damage to the samples during the preparation. For this limited dataset, total lipid contribution (assuming 70 % carbon content in lipids) to POC increased from epipelagic (7.7%) to bathypelagic (9.1%) zone.

Degradation of lipids as indicated by the Lipolysis Index (LI) values (Fig. 2d) was highest at the first 30 m of depth where LI ranged from 0.24 to 0.38. Below 30 m, the LI values were very low (except at 2000 m depth) and ranged from 0.05 to 0.15. This LI distribution indicates a substantial lipid degradation from the surface to 30 m, whereas very low lipid degradation was observed from >30 m to 4800 m.

With the FT-ICR MS approach we monitor several thousand lipid elemental compositions for each sample collected. In the negative ionization mode, 4,908 different lipids were detected (excluding contributions from 13C and other heavy nuclide-containing species) among which 1,254 elemental compositions matched lipid elemental compositions reported in the Nature Lipidomics Gateway database (<http://www.lipidmaps.org/>). In the positive ionization mode, 3,596 monoisotopic lipid compounds were detected for which 609 matched known lipid elemental compositions. These observations indicate the level of current documentation of marine lipids and highlight the limitation of lipid characterization techniques used to generate the database.

Here we present qualitative FT-ICR MS characterization of simple acylglycerols, where tri- (TG) and di-acylglycerols (DG) (both observed with even and odd numbers of carbon atoms) and monoacylglycerols (MG) (observed with even carbon numbers only) are detected in the positive ionization mode and free fatty acids (FFA, both even and odd carbon numbers, unsaturated (UFA), branched (BrFA) and saturated (SAFA)) are detected in the negative ionization mode. Although many oxidative forms of TG and FFA were also detected, those data are not presented to avoid any misinterpretation of lipidome due to the possible lipid oxidation during extraction and/or storage.

The depth related change of the cumulative FT-ICR MS signal magnitude of total lipids and that for TGs, DGs, MGs and FFA and their relative abundance changes are shown in Fig. 3. The signal magnitude for each compound is proportional to its solution phase concentration although response factors for each compound are not known due to the unavailability of standards for all compounds observed. Abundance differences for the observed compounds at various depths were compared by normalization of the total signal at each depth to the lowest cumulative signal obtained at a given depth. In general the highest abundance values were measured in the euphotic zone. Unsaturated fatty acids showed the largest decrease (up to 420-fold) compared to BrFA (up to 48-fold) and TG (up to 16-fold). There was no significant decrease in the relative abundance of MG and SAFA with ocean depth.

The contribution of triacylglycerol (TG and oddTG), DG, MG and fatty acid (UFA, BrFA and SAFA) signals to the total lipid signal are shown in Figs. 2c and f. The depth-dependent lipid abundances indicate that these lipid classes contribute most greatly to the total lipid signal in the euphotic zone with the exception of SAFA whose contribution substantially increases with depth, up to 39%. The contribution of TG and DG to total lipids was substantial (up to 11.5 and 5.2%) at the surface productive depths (Fig. 3e). BrFA contributed 3.0-4.7% in epipelagic and 1.0-2.0% in the depths below epipelagic zone. The lowest contribution was observed for UFA, being 0.6-1.2% in epipelagic and 0.02% in bathypelagic (1000-4000 m depth) zone.

We observe that some lipids are observed primarily in the euphotic zone, or meso- to abyssopelagic (200-4800 m depth) zone, whereas other lipids maintain concentration across the entire water column. Table 1 shows acyl DBE (DBE, double bond equivalents which is the number of molecular rings plus double bonds to carbon) and carbon number content of the most abundant lipids observed in the epipelagic and below epipelagic depths and their contribution to the lipid class.

The lipid class with the most observed compounds for the whole ocean depth profile was that of TG with 99 TG compounds detected. The DG and oddTG lipid classes were slightly less diverse with 82 and 76 molecular formulae. Only 4 different MG formulae were detected. The molecular diversity of fatty acids was reflected in 8 UFA, 13 BrFA and 22 SAFA molecular formulae. The molecular diversity *i.e*. the number of assigned elemental compositions of observed lipids across ocean depths is presented in Fig. 4. The highest molecular diversity was observed for the euphotic zone with the exception of SAFA for which increased diversity was observed with increased depths.

The use of FT-ICR MS allows qualitative characterization for detected lipid signals by defining mass, average number of carbon atoms in the acyl chain of tri-, di-, and monoacylglycerols and carbon atoms in fatty acids, together with double bond equivalents The average molecular mass (Mw) of the observed lipids is shown in Fig. 5. Average molecular mass for oddTG and TG decreased from the epipelagic zone (810 and 801 Da, respectively) to 4800 m depth (780 and 715 Da, respectively). Mw of DG varied in a range between 626 Da at the surface and 592 Da in the deep Atlantic (4800 m). Only four formulae were detected for MG and the Mw remains constant throughout the whole water column. The average number of C-atoms in acyl chain of TG decreased from the epipelagic layer (15.5-16.1) to 4800 m depth (13.9) (Appendix Fig. 1) while the average acyl carbon number of oddTG (15.3 to 16.3) (Appendix Fig. 2) remained essentially consistent with depth. Longer acyl chains were detected for DG, being 18.1-18.4 at epipelagic and 16.6−17.2 at abyssopelagic depths (Appendix Fig. 3). Average acyl carbon number of MG was constant at 18.5 from the surface to 3500 m and decreased to 18.0 below 3500 m.

We observed an interesting feature for FFA where the average molecular weight increase with depth (Fig. 5c) (Fig. 5d and Appendix Figs. 4-6). The average molecular weight of UFA, BrFA and SAFA ranged from 289 to 366 Da, 295 to 352 Da, and 291 to 375 Da, respectively.

The degree of unsaturation of the observed lipids is shown as acyl double bond equivalent content, is shown in Figure 6. Observed lipids generally show higher levels of acyl unsaturation at surface productive depths as opposed to the deep ocean. The epipelagic zone average unsaturation decreased in the order TG (DBE 3.5-4.7) > oddTG (DBE 2.1-4.7) > UFA (DBE 3.4-3.7) > DG (DBE 3.1-3.9) > BrFA (DBE 1.3) = MG (DBE 1.3).

**4. Discussion**

Using FT-ICR MS and Iatroscan we present details of oceanic particulate lipid composition that has not been previously achieved. We discuss the cycling of suspended simple lipids that contain only C, O and H in the molecular formulae, through the qualitative and quantitative description of their change through the ocean depths. Lipid analysis with the Iatroscan technique was performed to reveal lipid class concentrations and ESI FT-ICR MS provides lipid characteristics such as degree of unsaturation and the extent of alkylation for observed lipids. Molecular formulae derived directly from FT-ICR MS measured m/z values are tentatively assigned to given lipid classes using an in-house assembled lipid database derived from Lipid Maps to provide depth-related lipid class distribution. Importantly, we observed similar water column distribution patterns for the observed classes for the two methods employed.

We report depth-related cycling of triacylglycerols with even carbon number (TG) and odd carbon number (oddTG) and lipid degradation indices being di-, and monoacylglycerols (DG and MG, respectively) and free fatty acids (FFA) including unsaturated fatty acids (UFA), branched fatty acids (BrFA) and saturated fatty acids (SAFA) that indicate lipid earliest stage of alteration (diagenesis) (Goutx et al., 2003). Triacylglycerols are storage lipids present in phytoplankton (Guschina and Harwood, 2009) and certain bacterial species (Goutx et al., 1990; Kalscheuer et al., 2007). Zooplankton may also synthesize TG as storage compounds (Lee, 1974), although zooplankton mainly store wax esters that serve as long-term metabolic reserves (Kattner and Hagen, 2009). Fatty acids in zooplankton have an even number of C-atoms (Kattner and Hagen, 2009), reflecting their diet i.e. feeding on primary producers that produce FA with even number of C-atoms. Unlike phytoplankton, bacterial metabolic pathways synthesize FA with odd-numbered, branched trans-unsaturated and cyclopropyl fatty acids. As a consequence 15:0, 17:0, 10-methyl-16:0, iso- and anteiso-branched saturated and monounsaturated have been used as bacterial fatty acid markers (Harkewicz and Dennis, 2011). Odd-chain FFA, DG and MG are not discussed explicitly as they are degradation products of triacylglycerol. We consider oddTG as an indicator of bacteria, although odd-chain FA are also biosynthesized by other organisms (Dalsgaard et al., 2003 and references therein).

The concentration of total lipids that we observed (3.0 to 24.9 μg L-1) are within the ranges published for the eastern Atlantic (3.2 to 29.4 μg L-1, Gašparović et al., 2014), and lower than those reported for the coastal regions of the NW Atlantic (28–58 µg L–1, Parrish et al., 1988), Gulf of Mexico (9-70 µg L–1, Kennicutt and Jeffrey, 1981) and west Mediterranean Sea (3–84 μg L-1, Gérin and Goutx, 1994).

Samples from different depths reflect variable periods relative to the original synthesis of the lipid at the surface productive layer. As the Niskin bottle collects a mixture of fast, slow and non-sinking particles, it should be stressed that different sinking particles are differentially prone to degradative processes. Namely, slow-sinking particles bear a stronger signature of bacterial degradation than do the fast-sinking particles (Wakeham et al., 2009). The compositional difference between fast-sinking and slow-sinking particles shows heterogeniety within the particle ﬁeld, an observation that implies limited material exchange between the two pools (Wakeham et al., 2009). The main lipid producers in the surface layer are phytoplankton (Gašparović et al. 2014) while mainly bacteria and to a much lesser degree protozooplankton, and mesozooplankton contribute to the lipid pool at the bathypelagic depths (Yamaguchi et al., 2002). Plankton biomass at the ocean depths below 1000 m ranges 0.4–1.7 µg C l-1 (Yamaguchi et al., 2002). Knowing that the lipid content of marine bacteria is low varying from 1.7% to 7.3% of organic carbon (Goutx et al., 1990) and that carbon from particulate lipids at depths below 1000 m in our samples ranged from 2.1 to 7.3 µg C l-1 (average 3.9 µg C l-1) one can assume that roughly 1% of the sampled lipids at the greatest depths originated from living plankton.

We also evaluate the possible contribution of living planktonic lipids to total measured lipids in the epipelagic zone. To do this we have converted Chl *a* to phytoplankton carbon by using conversion formulae:

Cphyto (μgC l-1) = 33.7\*Chl *a* (μgC l-1) + 9.8 (Graff et al., 2015)

Average marine plankton contain roughly 19% lipids (Emerson and Hedges, 2008). Using calculations of Marić et al. (2013) we recalculated the quantity of Iatroscan-detected lipids from phytoplankton, as a main lipid producer in the epipelagic layer:

 Lipidphyto (μgC l-1) = Cphyto (μgC l-1) × 0.19,

and

Lipidphyto (%) = (Lipidphyto (μgC l-1) x 100)/Ctotal lipid (μgC l-1).

We estimated that on average 66% of measured lipids in the epipelagic originated from living phytoplankton. This suggests that a large portion of the epipelagic lipids are in non-living organic matter.

Depth-related total lipid concentration follows the well-known plankton distribution, being the highest at the surface productive layer and decreasing with depth, which shows major production at surface and a decrease both as a consequence of decreased contribution of living plankton with depth together with lipid transformation and degradation processes as depth increases. However, increased contribution of total lipids to POC between epipelagic and bathypelagic zones indicates depth related total lipid selective preservation. The depth related distribution is preserved for all observed lipids and indicates major production at the surface (TG and oddTG) but also significant lipid alteration (indicated by DG, MG and FFA) in the surface productive layer; an observation that illustrated high lipid degradation in the epipelagic zone. This is also confirmed by the highest Lipolysis index observed there (Fig. 2d). Regardless, the highest concentration decrease of all lipid classes and POC occurred between 50 m (1% of surface irradiance) and 100 m depth indicating the most intensive OM degradation processes took place just below photic zone. The mesopelagic layer or “twilight zone” (100−1000 m) is characterized by the most dynamic biological and physical processes (Lee et al., 2004) where rapid biological consumption and remineralization of carbon reduce the efficiency of sequestration (Buesseler et al., 2007). It is shown that processes in the “twilight zone” are site specific and depends on pelagic food web structure, the proportion of faecal pellets versus phytoplankton aggregates, the fraction of export associated with ballast minerals and their sinking rates, water temperature, and C demand of the mesopelagic heterotrophic communities (Buesseler et al., 2007).

Plankton energy-storage compounds, TG are found in particulate matter as a part of living cells or are released through exudation, lysis and grazing. Our results show that TG compounds comprise a diverse class with a variety of fatty acid compositions, especially at epipelagic depths (on average 81 molecular formulae per depth measurement). Triacylglycerols cycling through the ocean depths cause substantial concentration decrease at mesopelagic depths (Figs. 1a, 2a and b) after which the TG concentration remains relatively constant until the deep ocean. Triacylglycerol molecular transformation throughout the ocean depth profile is reflected by the decrease in the number of TG compounds, average acyl carbon number (15.8 at epipelagic to 14.2 at abyssopelagic zones) (and consequently molecular mass; 788 Da at epipelagic to 731 Da at abyssopelagic zones) and average acyl chain unsaturation (DBE=4−5 at epipelagic layer and DBE= 1−2 at depths below 200 m). Obviously between 200 and 300 m depth major TG transformation occurred with highly altered TG molecules found below 200 m.

Our results suggest that TG with one unsaturation in acyl chain survive until abyssopelagic depths, with a possible contribution from living organisms, where TG contributed on average 3.2% to total FT-ICR MS signal. High TG molecular diversity, on average 36 molecular formulae per sample, is detected even in the abyssopelagic zone. This suggests that TGs are important components of the particle ﬂux in the oceans. This is in line with data from Wakeham (1985) and Goutx et al. (2000). Although Afi et al. (1996) suggested that TG are labile compounds preferentially hydrolysed through bacterial activity when compared to other lipid classes and Yoshimura et al. (2009) showed in a model experiment that TG rapidly decrease during early diagenesis, our results suggest that TG fraction with high unsaturation is prone to degradation while the saturated TG are stable even at high depths (Fig. 6a) and can contribute to carbon sequestration.

The unsaturation of oddTG is lower than for TG which is not surprising knowing that bacteria mainly synthesize saturated and monounsaturated FA (Harkewicz and Dennis, 2011). Changes of oddTG are characterized by a modest decrease in the abundance (Fig. 3a and c) and the number of compounds (Fig. 4a) with depth, invariability in molecular mass and acyl carbon number over depths with an average Mw of 794 Da (Fig. 5a) and an average acyl carbon number of 15.7 (Fig. 5b), and substantial decrease in the unsaturation from epipelagic (average DBE = 3.9) to abyssopelagic (DBE = 1.4) depths (Fig. 6a). However, we do not have an explanation for such high unsaturation of oddTG at the epipelagic zone knowing that bacteria that produce polyunsaturated fatty acids are inhabiting relatively unusual environments including the low-temperature deep sea and the intestines of sea fish (Okuyama et al., 2007). Our observations for oddTG and TG composition imply that both lipid classes are subjected to similar chemical and bacterial action with enzymatic hydrolysis being particularly important (Killops and Killops, 2005). However unlike TG, the contribution of oddTG to the total lipid does not significantly change with depth (although different oddTG were observed at the surface than in the abyssopelagic zone, with a high probability that living bacteria oddTG contributed to total lipids in the abyssopelagic zone).

The diversity of DG significantly decreased from the euphotic zone to the mesopelagic and into the abyssopelagic (Fig. 4a) depths. The relative abundance of DG decreases about 8-fold (Fig. 3c). In addition, a modest decrease in acyl carbon number (18.2 in epipelagic and 16.9 in abyssopelagic zones) and a substantial decrease in the acyl DBE (an average of 3.6 at epipelagic and 0.6 in abyssopelagic zones) were also observed for DG. Four MG molecules were detected in the whole water column suggesting their high susceptibility to degradation. The nature of the observed MG indicates that only saturated MG are stable i.e. not prone to further degradation in the Northeast Atlantic.

Depth-related relative abundance changes of reported lipid classes (Fig. 3e) show the highest reduction in relative abundance for UFA in the water column in comparison to other observed lipids. This is not surprising as unsaturated organic substances are the most reactive compounds that undergo abiotic (photodegradation) or biotic (enzymatic) degradation. Selective and efficient removal of polyunsaturated fatty acids with depth was observed in other studies (Wakeham et al., 1997; Loh et al., 2008). Only 1−8 different UFAs were observed for the whole water column (Fig. 4b) and the degree of unsaturation substantially decreased from epipelagic to bathypelagic depths, while no UFA were detected in the abyssopelagic zone (Fig. 6b). UFA at greater depths might be of bacterial origin and we suggest that 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).

We found that at epipelagic depths three UFA dominated the UFA pool (Table 1). Among the observed unsaturated C16 fatty acids, 16:4 and 16:2 show the highest abundance which serve as a specific marker of diatoms, together with 16:0 (Dalsgaard et al., 2003), which was also found abundant in our samples. Also the observed long-chain, highly-unsaturated FA C28:8 have been previously identified in several species of dinoflagellates (Van Pelt et al., 1999, Mansour et al., 1999). The presence of these UFAs strongly suggests that diatoms and dinoflagellates were important contributors to the total lipid pool in the epipelagic zone at PAP. This is in line with the classic North Atlantic bloom scenario, in which diatoms bloom in spring and are then succeeded by dinoflagellates and other small phytoplankton in summer (Henson et al., 2012).

An observation of some significance is that the bacterial markers BrFAs are efficiently removed from the water column (Figs. 3d and e). Monounsaturated BrFAs C16:1 and C18:1, dominant throughout the whole water column (Table 1) are found to be the major fatty acids, together with C16:0, in many marine bacteria (Oliver and Colwell, 1973). The low molecular diversity of BrFA did not change significantly over the whole water column (Fig. 4b), as well as unsaturation (Fig. 6b). This would suggest that these BrFA are regular part of bacterial lipids or less prone to degradation.

Unlike other observed lipids, the contribution of SAFA to total lipids increased with ocean depth (Fig. 3f). Their cycling is characterized by increased number of different SAFA molecules together with longer chain length and molecular mass with increasing depth. This clearly shows that diagenesis of lipids includes synthesis of transformation-resistant SAFA of increasing molecular weight with depth. The dominance of C14:0, C16:0 and C18:0 SAFA in the water column (Table 1) suggest their *in situ* origin given that markers for terrestrial origin are long chain C22:0 to C32:0 SAFA (Meziane et al., 1997; Budge et al., 2001). Domination of SAFA in the fatty acid pool has also been found in the Sargasso Sea and North Central Paciﬁc (Loh et al., 2008).

**5. Conclusion**

The cycling of organic carbon in the marine environment is a key process in the global carbon cycle. Although autotrophic production occurs in the sunlit surface waters, sinking provides the major pathway for transportation of particulate organic carbon from surface waters to the ocean depths and sediments. Organic matter molecular level characterization of lipids produced in the surface productive layer and transported to deep ocean reflects an important consideration of oceanic carbon sequestration.

The use of FT-ICR MS provides molecular level characterization of suspended particulate lipids collected in the Northeast Atlantic. Here, we analyzed for the first time particulate lipid material by FT-ICR MS to provide elemental composition of free fatty acids, tri-, di- and monoacylglycerols that served as diagnostic markers for their transformation processes and their preservation potential through the water column. The vertical distribution of observed lipids reﬂects their production in the surface waters and their degradation as the particles sink. The most reactive layer is between 200-300 m depth where the majority of observed lipids are substantially degraded and/or altered. There is richness of different lipid molecular formulae, and especially of TG, oddTG and DG in the surface productive layer (epipelagic zone), while observed lipid molecular variability is reduced, except for SAFA, until abyssopelagic depths.

The depth-related changes of observed lipids from the Northeast Atlantic are selective with the general observation of decreasing concentration and changed molecular characteristics with increasing water depth. The main depth-related lipid molecular characteristics change is the substantial loss of unsaturation with depth. Consequently, the deep Atlantic is rich in SAFA and in saturated or monounsaturated TG, oddTG, DG and BrFA demonstrating that saturated lipids are not prone to degradation and are therefore important organic compounds for transport of carbon to depth and in the functioning of the biological carbon pump.

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**Figure captions**

Fig. 1. Depth distribution of (a) temperature (T) (b) salinity (S) and (c) chlorophyll fluorescence (as a measure of Chl *a* concentration). Marked are depths of lipid samples (squares).

Fig. 2. Depth distribution of (a) Iatroscan determined total lipid (squares), triacylglycerol (TG) (circles), diacylglycerol (DG) (upward triangles), monoacylglycerol (MG) (diamonds) and free fatty acid (FFA) (stars) concentrations; (b) the contribution of TG (circles), DG (up triangles), MG (diamonds) and FFA (stars) to total lipids; (c) particulate organic carbon (POC); and (d) Lipolysis Index (LI).

Fig. 3. Cumulative signal magnitude (a, d), and relative abundance (b, e) and the contribution of each lipid class to the total lipid signal (c, f) as a function of sampling depth derived from positive (upper panels) and negative (lower panels) ion ESI FT-ICR MS. Lipid classes are denoted as (a-c) TG (diamonds), oddTG (stars), DG (right triangles), MG (down triangles), and (d-f) unsaturated fatty acids (UFA) (up triangles), branched fatty acids (BrFA) (squares) and saturated fatty acids (SAFA) (circles).

Fig. 4. Number (No.) of lipid elemental compositions that correspond to given lipid classes as a function of sampling depth for (a) TG (diamonds), oddTG (stars), DG (right triangles) and MG (down triangles); and (b) UFA (up triangles), BrFA (squares) and SAFA (circles).

Fig. 5. Average molecular mass (Da) (a, c); and acyl carbon number (No.) (b, d) as a function of sampling depth for (a, b) TG (diamonds), oddTG (stars), DG (right triangles), MG (down triangles), and (c, d) unsaturated fatty acids (UFA) (up triangles), branched fatty acids (BrFA) (squares) and saturated fatty acids (SAFA) (circles). The average carbon number per acyl chain for TG, oddTG, DG and MG are calculated by subtracting three carbon atoms (for the backbone) from the total carbon number and dividing by the number of acyl chains in the lipid molecule.

Fig. 6. Double bond equivalents (DBE) as a function of sampling depth of (a) TG (diamonds), oddTG (stars), DG (right triangles) and MG (down triangles), and (b) UFA (up triangles), and BrFA (squares). Acyl DBE for TG and oddTG are calculated by subtracting 3 DBE from the total DBE calculated from the molecular formula. Acyl DBE for DG and MG are calculated by subtracting 2 and 1 DBE, respectively from the total DBE.

Table 1. The elemental composition, average carbon number and average double bond equivalents (DBE) of the acyl groups, and their contribution to the class for the most abundant lipids found at epipelagic (0-200 m) and below epipelagic (200-4800 m) zone. Bold formulae are observed dominant in the whole water column.

|  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- |
|  | Elemental composition | Acyl DBE | Acyl C number  | Contribution to the lipid class (%) | Elemental composition | Acyl DBE | Acyl C number | Contribution to the lipid class (%) |
| 0-200 m | 200-4800 m |
| TG | **C49H92O6****C51H96O6**C53H100O6 | 111 | 15.31616.7 | 14-25 | C9H14O6**C49H92O6****C51H96O6**C57H104O6 | 0113 | 315.31618 | 15-55 |
| oddTG | **C48H92O6****C50H96O6****C50H94O6** | 001 | 1515.715.7 | 15-25 | **C48H92O6****C50H96O6****C50H94O6** | 001 | 1515.715.7 | 21-46 |
| DG | C37H64O5C41H68O5 | 46 | 1719 | 11-23 | C35H68O5C37H72O5C39H76O5 | 000 | 161718 | 16-60 |
| MG | **C19H38O4****C21H42O4****C23H46O4** | 000 | 161820 | 85-93 | **C19H38O4****C21H42O4****C23H46O4** | 000 | 161820 | 92-100 |
| UFA | C16H28O2C16H24O2C28H40O2 | 248 |  | 89-90 |  |  |  |  |
| BrFA | **C16H30O2****C18H34O2**C22H32O4 | 116 |  | 90-94 | **C16H30O2****C18H34O2**C24H48O3 | 110 |  | 74-93 |
| SAFA | C14H28O2**C16H32O2****C18H36O2** | 000 |  | 88-93 | **C16H32O2****C18H36O2** | 00 |  | 72-92 |

Appendix:

**Free fatty acids, tri-, di- and monoacylglycerol production and depth-related cycling in the Northeast Atlantic** by Blaženka Gašparović, Abra Penezić, Richard S. Lampitt, Nilusha Sudasinghe and Tanner Schaub

This section includes the data used to construct Fig. 2 (Appendix Table 1) as well as relative intensity contribution of the average acyl carbon number of triacylglycerols (TG) to total TG, of odd triacylglycerols (oddTG) to total oddTG, of unsaturated fatty acids (UFA) (DBE>0) having acyl carbon number between C12 and C28 to total UFA, Relative intensity contribution of branched fatty acids (BrFA) having acyl carbon number between C14 and C43 to total BrFA, and of saturated fatty acids (SAFA) (DBE=0) having acyl carbon number between C9 and C37 to total SAFA (Appendix Figs. 1-6, respectively).

Appendix Table 1. Iatroscan determined total lipid, triacylglycerol (TG), diacylglycerol (DG), monoacylglycerols (MG) and free fatty acid (FFA) concentrations, the contribution of TG, DG, MG and to total lipids, particulate organic carbon (POC) and Lipolysis Index (LI) data.

|  |  |  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| Depth | Lipid | TG | TG | DG | DG | MG | MG(%) | FFA | FFA(%) | POC | LI |
| (m) | (µg L-1) | (µg L-1) | (%) | (µg L-1) | (%) | (µg L-1) | (%) | (µg L-1) | (%) | (µg L-1) |  |
| 2 | 16.23 | 0.34 | 2.12 | 0.21 | 1.27 | 0.09 | 0.57 | 1.72 | 10.57 | 141.0 | 0.28 |
| 5 | 18.43 | 0.45 | 2.42 | 0.25 | 1.33 | 0.04 | 0.23 | 2.90 | 15.74 |  | 0.38 |
| 15 | 11.13 | 0.37 | 3.31 | 0.27 | 2.44 | 0.06 | 0.52 | 0.87 | 7.82 |  | 0.24 |
| 25 | 20.53 | 0.51 | 2.49 | 0.39 | 1.88 | 0.04 | 0.17 | 2.33 | 11.35 | 202.9 | 0.28 |
| 30 | 24.28 | 1.91 | 7.88 | 0.23 | 0.95 | 0.13 | 0.52 | 3.49 | 14.35 | 154.3 | 0.28 |
| 50 | 10.61 | 0.39 | 3.64 |  |  | 0.03 | 0.30 | 0.12 | 1.09 |  | 0.05 |
| 100 | 4.28 | 0.16 | 3.73 |  |  | 0.00 | 0.11 | 0.25 | 5.88 | 65.1 | 0.12 |
| 200 | 4.49 | 0.13 | 2.79 | 0.04 | 0.80 |  |  | 0.05 | 1.19 | 57.9 | 0.05 |
| 300 | 5.22 | 0.14 | 2.59 | 0.09 | 1.78 | 0.02 | 0.40 | 0.23 | 4.40 | 32.3 | 0.10 |
| 400 | 4.93 | 0.14 | 2.77 | 0.07 | 1.48 | 0.01 | 0.20 | 0.24 | 4.80 | 28.0 | 0.11 |
| 600 | 4.42 | 0.14 | 3.11 | 0.05 | 1.07 | 0.01 | 0.28 | 0.16 | 3.60 | 62.1 | 0.09 |
| 800 | 7.32 | 0.17 | 2.26 | 0.06 | 0.76 | 0.02 | 0.26 | 0.16 | 2.13 | 82.7 | 0.09 |
| 1000 | 7.19 | 0.19 | 2.61 | 0.08 | 1.09 | 0.01 | 0.20 | 0.27 | 3.76 | 52.2 | 0.12 |
| 1500 | 5.63 | 0.08 | 1.47 | 0.02 | 0.43 | 0.01 | 0.14 | 0.04 | 0.77 | 29.0 | 0.05 |
| 2000 | 10.31 | 0.16 | 1.52 | 0.30 | 2.94 |  |  | 0.58 | 5.62 | 52.4 | 0.23 |
| 2500 | 4.55 | 0.13 | 2.76 | 0.12 | 2.64 | 0.02 | 0.42 | 0.22 | 4.84 | 38.4 | 0.15 |
| 3000 | 4.63 | 0.11 | 2.32 | 0.08 | 1.65 | 0.03 | 0.74 | 0.15 | 3.27 | 69.2 | 0.14 |
| 3500 | 2.97 | 0.05 | 1.75 | 0.06 | 1.88 | 0.02 | 0.63 | 0.14 | 4.61 | 46.4 | 0.13 |
| 4000 | 3.24 | 0.09 | 2.70 | 0.02 | 0.63 |  |  | 0.06 | 1.88 | 24.3 | 0.06 |
| 4500 | 6.42 | 0.11 | 1.64 | 0.06 | 0.94 | 0.01 | 0.23 | 0.28 | 4.44 |  | 0.13 |
| 4800 | 4.21 | 0.08 | 1.89 | 0.03 | 0.62 | 0.02 | 0.46 | 0.16 | 3.71 |  | 0.10 |



Appendix Fig. 1. Relative intensity contribution of the average acyl carbon number of triacylglycerols (TG) to total TG.



Appendix Fig. 2. Relative intensity contribution of the average acyl carbon number of odd triacylglycerols (oddTG) to total oddTG.



Appendix Fig. 3. Relative intensity contribution of the average acyl carbon number of diacylglycerols (DG) to total DG.



Appendix Fig. 4. Relative intensity contribution of unsaturated fatty acids (UFA) (DBE>0) having acyl carbon number between C12 and C28 to total UFA.



Appendix Fig. 5. Relative intensity contribution of branched fatty acids (BrFA) having acyl carbon number between C14 and C43 to total BrFA.



Appendix Fig. 6. Relative intensity contribution of saturated fatty acids (SAFA) (DBE=0) having acyl carbon number between C9 and C37 to total SAFA.