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

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ABSTRACT

We present the characterization 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 characterization. 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 analyzed, while free fatty acids were analyzed 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.



## 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,

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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 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 $C_{15}$ and $C_{17}$ fatty acids increase in relative abundance as particulate matter sinks. Long-chain $C_{37}$–$C_{39}$ alkenones of marine origin and long-chain $C_{20}$–$C_{30}$ 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. 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 (105 m). 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 PAP-SO 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 $CO_2$ in surface waters throughout the year, giving rise to a perennial $CO_2$ 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. 10 μg 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/) 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