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Differential response of fatty acid composition in the different lipid classes from particulate matter in a high arctic fjord (Kongsfjorden, Svalbard)

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ABSTRACT

Lipid classes and the respective fatty acid composition of natural particulate matter were studied on a seasonal basis in the Arctic fjord Kongsfjorden (Svalbard) during the early summer of 2006 and the spring, summer of 2007. Polar lipids were the major lipid class most of the times in 2006 and at all times in 2007. Among neutral lipids triglycerides were dominant. Polar lipids were divided into glycolipids (chloroplast membranes) and phospholipids (live cell membranes). Glycolipids were further divided into monogalactosyldiglycerides (MGDG), the major glycolipid, followed by digalactosyldiglycerides (DGDG) and Sulfoquinovosyldiglycerides (SQDG). In 2007, changes in both polar lipid constituents showed similar increasing trend from May to mid June but subsequently showed opposite trends from July to September. The seasonal pattern of particulate glycolipids was one of the low concentrations of MGDG in June followed by an increase between late June and September. SQDG exhibited a similar trend while DGDG displayed an opposing trend. In 2007, fatty acid composition of phospholipids, glycolipids and neutral lipids was dominated by saturated acids at all times followed by mono unsaturated acids and polyunsaturated acids with docosahexaenoic acid (DHA, 22:6n-3), eicosapentaenoic acid (EPA, 20:5n-3) and 18:5n-3 as major contributors. Glycolipid fatty acid pattern differed from that of phospholipids, showing a more important contribution of 18:5n-3. Neutral lipid composition differed from the other two classes by a larger contribution of 16:1n-5 and percentages of EPA, DHA, 18:5 and 18:4n-3 lower than in the two structural classes. Factorial correspondence analysis (FCA) of the fatty acid composition of all classes illustrated a seasonal transition in species composition and/or physiological states for the phospholipids and the different processes of chloroplast membrane adaptation in relation to taxonomic changes for the glycolipids. Neutral lipid changes were more complex because of the combined influence of growth rates, nutrient limitations and community shifts. The differential response of the different lipid classes is discussed in relation with the complex interactions between community structure, environmental adaptation and metabolic processes.

1. Introduction

Different taxonomic groups varying in size, physiological, ecological characteristics, and evolutionary origin constitute the assemblages of particles encountered in the natural marine environment. The presence of phytoplankton (autotrophs), protists (heterotrophs) and bacteria associated with detrital material has always been a challenge in the analysis of lipid and fatty acid flux through the pelagic food web (see Parrish et al., 1995; Reuss and Poulsen, 2002; Mayzaud et al., 2013). In most cellular systems lipid classes and their synthesis are separated into specific compartments: neutral lipids are storage molecules located in cytoplasm globules (Liu and Lin, 2001), glycolipids are part of photosynthetic membranes within the chloroplast system and phospholipids composed most of the other cell membranes. Hence, factors controlling lipid synthesis and fatty acid structure are specific for each class. For neutral lipids nutrient limitation is one of the key factors regulating accumulation since under limiting nitrogen supply excess synthesis of carbon is channeled towards lipids or carbohydrate storage (Shifrin and Chisholm, 1981). For glycolipids, light and photosynthetic activity are the most influential in relation to the need to adapt the chloroplast structure to the light condition either through the substitution of glycolipid classes or through fatty acid composition (Constantopoulos and Bloch, 1967; Mock and Kroon, 2002a,b). With regard to membrane lipids, growth rate and temperature are known to have large effects related to cell cycles and/or requirement to maintain optimal membrane fluidity (Saoudi-Helis et al., 1994).

The differential response of the distinct lipid classes and their respective fatty acid composition to changes in the physico-chemical environment provides information on the processes underlying physiological adaptations of the different taxonomic groups. Reuss and Poulsen (2002) and Mayzaud et al. (2013) emphasized the question

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of proper interpretation of fatty acid changes in natural plankton populations using total fatty acid patterns. In this study, we re-analyzed the seasonal changes of coastal arctic microplankton using specific lipid classes in order to clarify the processes at work.

Within the context of the Kongsfjorden phytoplankton production the 2006 and 2007 periods were characterized by abnormally high temperature. As a result, the absence of arctic waters and the dominance of transformed Atlantic waters late spring explained the lack of a spring diatom bloom and the occurrence of a *Phaeocystis pouchetii* bloom (Mayzaud et al., 2013). The summer communities were characterized by the dominance of prymnesiophytes, naked ciliates and cryptophytes. Significant contribution of diatom biomass was observed only in the late summer–early fall (Mayzaud et al., 2013). Chlorophyll *a* concentrations showed similar range in both years, without high concentration in spring but with an increasing trend as summer developed.

The data presented in this paper are part of a larger study investigating the seasonal changes particulate matter lipids and the trophic interactions with zooplankton ("PRACEAL": Planktonic predators. Role and adaptive strategy in the control of Arctic ecosystem: importance of the lipid transfers).

2. Material and methods

An initial pilot study in summer 2006 was followed by a more detailed seasonal survey in 2007 in Kongsforden, Svalbard, Norway (78°57′N, 11°50′E). Samplings took place weekly between June 22nd and July 11th 2006 and between April 18th and September 25th 2007 at station KB3 (see Mayzaud et al., 2013, for station location). Sample collection as well as particle filtration and treatment have been fully described in Mayzaud et al. (2013). Briefly, water sampling was performed during daytime using a 12 L Niskin bottle, at various surface depths (2 to 5 m, depending on the sediment charge due to river run off). Vertical profiles of temperature, salinity, density and *in situ* fluorescence were measured with a CTD profiler equipped with a Seapoint chlorophyll fluorometer. Samples of 1 L of surface water were filtered on GF/F filters for measurements of particulate chlorophyll *a* using a Turner Design 10 fluorometer.

Particulate matter for lipid analysis was obtained after filtration of 60 L of seawater on chloroform extracted 14.2 cm diameter TCLP ($CHCl_3$:MeOH 2:1) filters. Filters were extracted according to the method of Bligh and Dyer (1959). After solvent evaporation at high vacuum, the extracted lipids were weighed in tarred vials on a precision balance (±10 μg) to evaluate the content of total lipids. The extracts were then placed under nitrogen at −80 °C until further analyses. Lipid classes were first separated using Extract-Clean SPE Silica 500 mg/8 mL Alltech column with the following solvent systems: neutral lipids with chloroform (6 column volumes), glycolipids with acetone (4 column volumes) and phospholipids with methanol (6 column volumes) followed by methanol/ammoniac 10% (90:10) (2 column volume). Collected fractions were dried on high vacuum and weighed with tared microvials (precision ± 10 μg). All operations took place under nitrogen. Validation of fraction purity and detection on TLC plates followed Mayzaud (1997).

In addition, lipid classes were quantified after chromatographic separation coupled with FID detection on an Iatroscan MK V (Ackman, 1981). Total lipid extracts were applied to Chromarods SIII using an SES A4100 Autopotter (1 μL) and analyzed in triplicate. Neutral lipids were separated using a double development procedure with the following solvent systems: n-hexane: benzene: formic acid 80:20:1 (by volume) followed by n-hexane: diethylether: formic acid 97:3:1.5 (v/v). Because of the quantities of lipids needed for analysis, glycolipid class separation was performed on a subset of samples with lipid concentrations high enough to achieve quantification and GLC determinations. After column isolation, the glycolipid fraction was dried on high vacuum, weighed and separated into classes on chromarods using chloroform:ethyl acetate:acetone:methanol:acetic acid:H_2O (60/12/15/16/3/3). Identification and calibration were achieved using commercial standards (Sigma).

An index indicating the degree of lipolysis (lipolysis index: LI) was computed following Parrish et al. (1995) using the equation: LI = (free fatty acid) / (total acyl lipids + products).

Fatty acid methyl esters of each lipid class were prepared with 7% boron trifluoride in methanol (Morrison and Smith, 1964). Gas liquid chromatography (GLC) of all esters was carried out on a 30 m length × 0.32 mm internal diameter quartz capillary column coated with Famewax (Restek) in a Perkin-Elmer XL Autolab gas chromatograph equipped with a flame ionization detector. The column was operated isothermally at 190 °C for methyl esters. Helium was used as a carrier gas at 7 psig. Injector and detector were maintained at 250 °C. Individual components were identified by comparing retention time data with those obtained from authentic and laboratory standards. In addition to the examination of esters as recovered, a part of all ester samples was completely hydrogenated and the products examined qualitatively and quantitatively by GLC. The level of accuracy is ±5% for major components, 1–9% for intermediate components and up to ±30% for minor components.

2.1. Statistical analyses

The significance of dissimilarity in fatty acid composition between classes or two depth layers was tested with an ANOSIM test (analysis of similarities, Clarke and Green, 1988) using PRIMER 5.

The covariation of fatty acids during the seasonal gradient was analyzed using factorial correspondence analysis (FCA) (Benzecri, 1973; Gower, 1987). The analysis was performed on a reduced data matrix transformed to relative frequencies and scaled so that each row (or column) can be viewed as a row (or column) of conditional probability distribution. The fatty acids were used to fill the matrix of variables and the different sampling dates were used as observations. Distances between fatty acid profiles were computed with a χ^2 metrics. This distance gives symmetry to the two sets of data (active variables and observations) so that each factorial axis associated to the cluster of variables corresponds to a factorial axis of the cluster of observations. Thus, it was possible to represent simultaneously descriptors and observations on the plane defined by the factorial axes. Graphs of projections retained those variables which displayed more than 1% total contribution to axes 1 and 2. Hierarchical clustering based on the factor score of all observations derived from FCA was based on Ward minimum variance method of clustering. Computation was made using the SPAD 5.5 software (Decisia).

To estimate the proportion of variance in the fatty acid composition of each class that can be explained by a linear combination of environment variables, a multivariate direct gradient analysis (redundancy analysis or RDA) was performed using the Canocco 4.5 software for windows (ter Braak and Smilauer, 2002).

3. Results

3.1. Neutral lipids and glycolipid classes

To clarify the origin of the dynamics observed at global scale (total lipids) and evaluate the possibility of periods of dominant physiological adaptation, lipid classes were quantified with two degrees of definition. Neutral lipids constituted of four main lipid classes: triglycerides, free fatty acids, sterols and diglycerides and polar lipids, which included two fractions: glycolipids and phospholipids. However their separation and quantification were possible only in June, July of 2006 and June, July, September of 2007.

The pattern recorded in 2006 showed a dominance of polar lipids except on July 7 when triglycerides were the major constituent suggesting either a strong change in population structure or in physiology (Fig. 1). No changes were detectable for the sterol and free fatty