Fatty acids Analysis

Using Bligh and Dyer (1959) method modified by Marty et al. (1994), lipids were extracted from freeze-dried samples using solvents mixture $CHCl_3$:CH_3OH:H_2O/1:2:0.9 (v:v:v), with a 5 minutes sonication. To extract the lipidic fraction, a mixture of $CHCl_3$:H_2O/2:2 (v:v) was added. Chloroform phase was removed from the extract using a speed-vac system. This phase contained total lipids (Fatty acids, sterols, chlorophyll...). The dried extract was then transesterified by 14% BF_3 in methanol under argon at 70 °C during 30 min. Transesterified lipids were extracted by hexane-diethylether (9:1; v:v) and the solvent removed by speed-vac. Separation of fatty acid methyl esters from total lipids was done on Sep-Pak silica cartridges using solvent of increasing polarity (Marty at al., 1994). Three fractions (F) were obtained: F_1, eluted by hexane, contained *n*-alkanes, F2 eluted by hexane and ethylacetate, contained fatty acid methyl esters (FAME) and F3, eluted by methanol, contained the more polar compounds.
FAME were then analysed by capillary gas chromatography (CGC) using a Hewlett Packard HP 5890 series II, with automatic split/splitless injector equipped with a DB wax, wall-coated open tubular column, 30 m long, 0.319 mm ID, He at 2 bar and operated from 120 to 240 °C at $2°C.min^{-1}$. Resolved compounds were identified by comparing their retention times to those of standards ran under the same conditions.

Quantification was determined by integration of peaks with the Turbochrom 3 analytical system and concentrations were calculated with respect to internal standard C19 fatty acid methyl ester added to the sample before extraction. Blanks were run throughout the analytical procedure to determine the presence of contaminants. The presence of C16 FAME was sometimes noticed but its maximum concentration was always below 5% of the smallest natural C16 peak.

Bligh E.G. and Dyer W.J., 1959. A lipid method of total lipid extraction and purification. Can. J. Biochem., 37, 911-917.

Marty J.C., Nicolas E., Miquel J.C. and Fowler S.W., 1994. Particulate fluxes of organic compounds and their relationship to zooplankton fecal pellets in the Northwestern Mediterranean sea. Mar. Chem., 46, 387-405.