Dataset name: **Fatty acids distribution and production**

|  |  |
| --- | --- |
| Parameters: | * **Total Fatty Acids (FA) concentrations** * **Neutral and Polar FA concentrations** * **FA classes (SAFA, BACT, PUFA, MUFA) concentrations** * **EPA (20:5n-3) and DHA (22:6n-3) concentrations** * **FA production** * **Taxonomy of microzooplankton** |

PROJECT TITLE: **MOBYDICK**

Oceanographic cruise: **MOBYDICK**

Start date: **18/02/2018**

End date: **27/03/2018**

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Geographic information: **Indian sector of the Southern Ocean**

Latitude: **49.5°S – 52.5°S**

Longitude: **67,0°E – 74.5°E**

Parameter supervisor: **Marine Remize**

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# OPERATIONS

## Sampling device(s)

Samples were obtained from In Situ Pumps (ISP) and CTD-rosette NISKIN bottles from 'OMICS' and 'NCP' casts.

**Table 1 : Parameters and sampling devices used**

|  |  |
| --- | --- |
| **Parameter** | **code of operation** |
| Total Fatty Acids (FA) concentrations | ISP, CTD\_OMICS\_P |
| Neutral and Polar FA concentrations | ISP, CTD\_OMICS\_P |
| FA classes (SAFAs, BACTs, PUFAs, MUFAs) concentrations | ISP, CTD\_OMICS\_P |
| EPA (20:5n-3) and DHA (22:6n-3) concentrations | ISP, CTD\_OMICS\_P |
| FA production | CTD\_NCP |
| Taxonomy of microzooplankton | ISP |

## List of stations sampled

**Table 2 : Stations and casts where the parameters were sampled**

|  |  |  |  |
| --- | --- | --- | --- |
| **Station ID** | **ISP #** | **rosette cast #** | **Cast ID TG** |
| M2-1 | ISP001 | CTD\_008 | CTD\_003 |
| M4-1 | ISP002 | CTD\_015 | CTD\_015 |
| M3 | ISP003 | CTD\_024 | CTD\_020 |
| M2-2 | ISP004 | CTD\_032 | CTD\_034 |
| M1 | ISP005 | CTD\_040 | CTD\_037 / CTD\_039 |
| M4-2 | ISP006 | CTD\_043 | CTD\_048 |
| M2-3 | ISP007 | CTD\_054 | CTD\_055 |
| M3-3 | ISP008 | CTD\_062 | CTD\_064 |

# INSTRUMENTS

* Total fatty acids (FA) concentrations, Neutral and Polar FA concentrations, FA classes (SAFAs, BACts, PUFAs, MUFAs) concentrations, EPA (20:5n-3) and DHA (22:6n-3):

Instrument Type: **Gaz chromatography - Flame Ionisation Detector (GC-FID)**

**Gaz chromatrography - Mass spectrometry (GC-MS)**

Manufacturer: **VARIAN**

Model: **CP4800**

Instrument Features / Calibration: Calibration with external and internal standards.

* FA production, 13C-labelled experiments (Units: µg.m-2.d–1):

Instrument Type: **Gaz Chromatography – Isotope Ratio Mass Spectrometry (GC-c-IRMS)**

Manufacturer: **Thermo Scientific**

Model: **Trace GC Ultra - Delta V plus**

Instrument Features / Calibration: Calibration with in-house standards and certified reference material.

# DESCRIPTION of PARAMETERS

## Parameters definition

Fatty Acids (FA) investigated in this work are carboxylic acids with an aliphatic chain containing between 13 up to 24 atoms of C, which are either saturated or unsaturated. The general formula for these compounds is: C:Yn-X, where C is the number of C atoms, Y the number of unsaturation and X the position of the first unsaturation.

According to their molecular structure, FA can be divided into different classes (Table 3) including Saturated FA with an even number of C atoms (SAFAs), Saturated FA with an uneven number of C atoms (BACTs), Mono-unsaturated FA (MUFAs), Poly-unsaturated FA (PUFAs) and Branched FA (BFAs). Among the PUFA class, EPA (20:5n-3) and DHA (22:6n-3) corresponds to omega-3 FA.

**Table 3 : Fatty acids definition and acronyms.**

|  |  |  |
| --- | --- | --- |
| Acronym | Definition | General Formula |
| SAFAs | *Saturated Fatty Acids* | C:0 with even C |
| BACTs | *Bacterial Fatty Acids* | C:0 with uneven C |
| MUFAs | *Monounsaturated Fatty Acids* | C:1n-X |
| PUFAs | *Polyunsaturated Fatty Acids* | C:Yn-X |
| n-3 | *Omega 3* | C:Yn-3 |
| n-6 | *Omega 6* | C:Yn-6 |
| BFAs | *Branched Fatty Acids* | iso/anteiso C:0 |
| Unknowns | *Unknowns Fatty Acids* |  |

## Sampling details

* Total Fatty Acids (FA) concentrations, Neutral and Polar FA concentrations, FA classes concentrations (SAFA, Bact-FA, PUFA, MUFA), EPA (20:5n-3) and DHA (22:6n-3) concentration

Samples for FA determination were obtained at variable depths between 20 and 300 m using large volume in-situ pumping systems (ISP cast) at all sampled stations. Suspended particulate matter were collected according to size with different filter configurations (1/50/300µm, 1/20/200 µm and 1/20/50 µm). All size fraction were subsampled for FA determination and immediately extracted using a 2:1 chloroform:methanol solvent. Lipid extracts were stored at –20°C. Additional samples for FA were obtained from seawater samples collected at CTD\_OMICS cast and at 3 depths (15 m, 40 m, and 100 m). Seawater samples (7 L) were filtered onboard and right after filtration, boiling water was added to the GF/F filter to stop lipase activity. GF/F filters were then extracted for lipids using 2:1 chloroform:methanol solvent extraction and stored at –20°C.

* FA production

Triplicateseawater samples (1 L) were obtained from the CTD-NCP cast at one depth. One sample was filtered right after collection and extracted for lipids in order to get the initial FA content. The two other samples were spiked with NaH13CO3–enriched solution and incubated onboard for 24 h under 50 % PAR light conditions. Incubation was stopped by filtration and samples were extracted for lipids.

* Microzooplankton taxonomy

Samples were collected only from the top filter of the lipid header of the ISP (200 µm). Subsampling was done after resuspension in filtered seawater and using a MOTODA splitting box. ¼ was recollected on a GF/F filter and then stored in a cryotube for further microscopical identification by Alice Delegrange (LOG Wimereux).

## Analytical procedure

The FA determination will include the following steps:

* 2:1 chloroform:methanol lipid extracts will be further separated into Neutral and Polar fraction using Solid Phase Extraction and with Neutral (98:2 Chloroform:methanol) and Polar (methanol) solvents.
* The two fractions (neutral and polar) will be then transmethylated to allow FA determination by GC-FID. Molecular identification and quantification will be performed according to retention time and using an internal standard of known composition (23:0) (Folch *et al.*, 1957 ; Soudant *et al.*, 1999).
* The identified FA will be grouped into different classes as detailed in Table 3.

For FA production, the same processing steps will be carried out. 13C-enriched abundance of FA in incubated samples will be determined by GC-c-IRMS. External standards and FA certified reference materials of known isotopic composition will be used for calibration.

## Units

* FA concentrations µg L–1
* FA production µg L–1 d–1
* Microzooplankton cell numbers L–1 for each taxon

## Sensor precision

N/A

## Post-cruise data analysis/treatment required

Post cruise sample treatment at home laboratory will include polar and neutral lipid separation and transmethylation. This procedure will be performed for all samples dedicated to FA determination.

## Estimated Date of Delivery

Analytical determination is expected to be done during 2019. The final database will be available by the end of 2019.

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Folch J., Lees M., Stanley G.H.S., 1957. A simple methods for the isolation and purification of total lipids from animal tissues. *The Journal of Biological Chemistry.*, **226**, 497–509.

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