Dataset name: **Flow cytometry analyses**

|  |  |
| --- | --- |
| Parameter: | * **Heterotrophic nanoflagellate abundances**
* **Autotrophic pico–naneukaryote abundances**
* **Bacterial (Bacteria + Archaea) abundances**
 |

PROJECT TITLE: **MOBYDICK**

Oceanographic cruise: **MOBYDICK**

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

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

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 **Observatoire Océanologique de Banyuls sur mer**

 **66650 Banyuls sur mer, France**

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

 Latitude: **49.5°S – 52.5°S**

 Longitude: **67,0°E – 74.5°E**

Parameter supervisor: **Ingrid Obernosterer**

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# OPERATIONS

## Sampling device(s)

Raw seawater samples were collected directly from the rosette bottles at all 8 stations. Eight shallow casts (CTD\_Stock, 0-1000 m, each depth) and 7 deep casts (CTD\_DeepStock,) were sampled.

## List of stations sampled

Shallow casts: M2\_1 (CTD-07), M4\_1 (CTD-13), M3 (CTD-23), M2\_2 (CTD-30), M1 (CTD-38), M4\_2 (CTD-42), M2\_3 (CTD-53), and M3\_3 (CTD-60).

Deep casts: M4\_1, M3, M2\_2, M1, M4\_2, M2\_3, and M3\_3.

**Table 1 : Sampling depths and bottle numbers for shallow casts**

|  |  |  |  |
| --- | --- | --- | --- |
| **Station M1** | **Stations M2-1, 2, and 3** | **Stations M3-1, and 3** | **Stations M4-1, and 2** |
| **bottle #** | **Depth** | **bottle #** | **Depth** | **bottle #** | **Depth** | **bottle #** | **Depth** |
| 21 | 25 m | 21 | 10 m | 21 | 25 m | 21 | 25 m |
| 19 | 50 m | 19 | 30 m | 19 | 50 m | 19 | 50 m |
| 17 | 75 m | 17 | 50 m | 17 | 75 m | 17 | 75 m |
| 15 | 100 m | 15 | 70 m | 15 | 100 m | 15 | 100 m |
| 13 | 125 m | 13 | 100 m | 13 | 125 m | 13 | 125 m |
| 11 | 150 m | 11 | 125 m | 11 | 150 m | 11 | 150 m |
| 9 | 175 m | 9 | 150 m | 9 | 175 m | 9 | 175 m |
| 7 | 200 m | 7 | 175 m | 7 | 200 m | 7 | 200 m |
| 5 | 250 m | 5 | 200 m | 5 | 250 m | 5 | 250 m |
| 3 | 500 m | 3 | 250 m | 3 | 500 m | 3 | 500 m |
| 1 | 1000 m | 1 | 500 m | 1 | 1000 m | 1 | 1000 m |

**Table 2 : Sampling depths and bottle numbers for deep casts**

|  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- |
| **station** | **cast #** | **bottle #** | **depth** |  | **station** | **cast #** | **bottle #** | **depth** |
| M4\_1 | CTD\_017 | 6 | 1000 m |  | M4\_2 | CTD\_044 | 14 | 1000 m |
| M4\_1 | CTD\_017 | 3 | 1900 m |  | M4\_2 | CTD\_044 | 11 | 2500 m |
| M4\_1 | CTD\_018 | 21 | 3000 m |  | M4\_2 | CTD\_044 | 7 | 3500 m |
| M4\_1 | CTD\_018 | 19 | 4000 m |  | M4\_2 | CTD\_044 | 2 | 4000 m |
| M3\_1 | CTD\_026 | 6 | 1000 m |  | M2\_3 | CTD\_052 | 20 | 25 m |
| M3\_1 | CTD\_026 | 1 | 1500 m |  | M2\_3 | CTD\_052 | 14 | 175 m |
| M2\_2 | CTD\_029 | 21 | 25 m |  | M2\_3 | CTD\_052 | 11 | 300 m |
| M2\_2 | CTD\_029 | 4 | 400 m |  | M2\_3 | CTD\_052 | 6 | 350 m |
| M2\_2 | CTD\_029 | 1 | 500 m |  | M2\_3 | CTD\_052 | 4 | 400 m |
| M1 | CTD\_035 | 8 | 1000 m |  | M2\_3 | CTD\_052 | 1 | 500 m |
| M1 | CTD\_035 | 5 | 2000 m |  | M3\_3 | CTD\_057 | 10 | 800 m |
| M1 | CTD\_035 | 1 | 2500 m |  | M3\_3 | CTD\_057 | 7 | 1000 m |
|  |  |  |  |  | M3\_3 | CTD\_057 | 1 | 1500 m |

# INSTRUMENTS

Instrument Type: **Flow cytometer**

Manufacturer: **BD Biosciences**

Model: **FACSCanto II**

Instrument Features / Calibration: Calibration beads

# DESCRIPTION of PARAMETERS

## Measurement details

For heterotrophic nanoflagellates (HNF) and pico-nanoeukaryotes each 4.5 mL seawater samples were fixed with 185 µL of glutaraldehyde (1% final concentration), and for bacterial abundances 1.44 mL of seawater were fixed with 60 µL glutaraldehyde (1% final concentration). Fixed samples were stored for 30 min at 4°C, shock frozen in liquid nitrogen and stored at –80°C until analysis.

## Analytical procedure

Heterotrophic nanoflagellates (HNF), phytoplankton and bacterial counts were performed with the FACSCanto II flow cytometer (Becton Dickinson) equipped with 3 air-cooled lasers : blue (argon 488 nm), red (633 nm) and violet (407 nm).

For HNF, staining was performed with SYBR Green I (Invitrogen—Molecular Probes) at 0.05% (v/v) final concentration for 15-30 min at room temperature in the dark (Christaki et al. 2011). The volume analyzed was around 1.5 ml at High Speed (around 150 µl/minute) for 10 minutes.

Bacterial cells were stained with SYBR Green I (Invitrogen - Molecular Probes) at 0.025% (vol/vol) final concentration for 15 min at room temperature in the dark (Obernosterer et al. 2008). Stained bacterial cells were discriminated and enumerated according to their right-angle light scatter (SSC) and green fluorescence measured at 530/30 nm. In a plot of green fluorescence versus red fluorescence, we were able to distinguish photosynthetic prokaryotes from non-photosynthetic prokaryotes. The speed of analysis was adapted to cell concentration, usually 1 minute at low speed, around 15-20 µl.

Phytoplankton cells were analysed according to their natural fluorescence (chlorophyll a and phycoerythrin). The volume analyzed was around 0.75 ml at High Speed (150 µl/minute) for 5 minutes.

The subsequent cell concentration was determined from the flow rate, which was calculated with TruCount beads (BD biosciences).

## Units

Cells/ml

## Sensor precision

N/A

## Post-cruise data analysis/treatment required

N/A

## Estimated Date of Delivery

End of 2018.

# BIBLIOGRAPHY

Christaki U., Lefèvre D., Georges C., Colombet J., Catala P., Courties C., Sime-Ngando T., Blain S., Obernosterer I., 2014. Microbial food web dynamics during spring phytoplankton blooms in the naturally iron-fertilized Kerguelen area (Southern Ocean). *Biogeosciences*, **11**, 6739-6753.

Obernosterer I., Christaki U., Lefèvre D., Catala P., Van Wambeke F., Lebaron P., 2008. Rapid bacterial mineralization of organic carbon produced during a phytoplankton bloom induced by natural iron fertilization in the Southern Ocean. *Deep Sea Research Part II: Topical Studies in Oceanography*, **55**(5-7), 777-789.