Dataset name: **Molecular eukaryotic diversity**

|  |  |
| --- | --- |
| Parameters: | * Paired-end high-throughput sequencing data (2x 300bp) of eukaryotic microbial communities: *UC01-Amplicon-LOG\_R1\_001.fastq.gz, UC01-Amplicon-LOG\_R2\_001.fastq.gz*
* Sequences of ASVs (amplicon sequencing variants): *ASVs\_seq.fa*
* Table with taxonomic assignments of ASVs: *ASVs\_taxonomy.txt*
* Table with sequence counts of ASVs in all samples: *ASVs\_counts.txt*
* Table with characteristic parameters of each sample: *18Smetadata.txt*
 |

PROJECT TITLE: **MOBYDICK**

Oceanographic cruise: **MOBYDICK**

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

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

Project manager: **Bernard Quéguiner** bernard.queguiner@mio.osupytheas.fr

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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: **Urania Christaki**

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# OPERATIONS

## Sampling device(s)

*Niskin bottles from 4 depths (10, 60, 125 and 300m depth), Phytonets 1-14 (125 m depth to surface), Bottlenets (variable depth)*

## List of stations sampled

*M2\_1, M4\_1, M3, M2\_2, M1, M4\_2, M2\_3, M3\_2*

*(CTD- Omics T, Phytonets, Bottlenets)*

|  |  |  |
| --- | --- | --- |
| ***Station*** | **Sample** | ***Depth*** |
| *M2.1* | *Phytonet\_001* | 0-125m |
| *M2.1* | *Phytonet\_002* | 0-125m |
| *M2.1* | *CTD\_007 - Bottlenet* | 100-450m |
| *M2.1* | *CTD\_009 - Niskin bottle* | 10, 60, 125, 300m |
| *M4.1* | *CTD\_011 - Niskin bottle* | 10, 60, 125, 300m |
| *M4.1* | *Phytonet\_003* | 0-125m |
| *M4.1* | *Phytonet\_004* | 0-125m |
| *M4.1* | *CTD\_013 – Bottlenet* | 125-500m |
| *M4.1* | *CTD\_017 – Bottlenet* | 150-1900m |
| *M4.1* | *CTD\_018 - Bottlenet* | 1900-4000m |
| *M3.1* | *Phytonet\_005* | 0-125m |
| *M3.1* | *CTD\_21 – Niskin bottle* | 10, 60, 125, 300m |
| *M3.1* | *CTD\_21 – Bottlenet* | 60-125m |
| *M3.1* | *Phytonet\_006* | 0-125m |
| *M3.1* | *CTD\_24 – Bottlenet* | 124-500m |
| *M3.1* | *CTD\_26 - Bottlenet* | 500-1500m |
| *M2.2* | *CTD\_27 - Niskin bottle* | 10, 60, 125, 300m |
| *M2.2* | *CTD\_27 - Bottlenet* | 100-150m |
| *M2.2* | *Phytonet\_007* | 0-125m |
| *M2.2* | *CTD\_30 – Bottlenet* | 125-400m |
| *M1.1* | *CTD\_35 – Bottlenet* | 500-2500m |
| *M1.1* | *CTD\_36 – Niskin bottle* | 10, 60, 125, 300m |
| *M1.1* | *CTD\_36 – Bottlenet* | 60-125m |
| *M1.1* | *Phytonet\_008* | 0-125m |
| *M1.1* | *CTD\_038 – Bottlenet* | 125-500m |
| *M4.2* | *Phytonet\_009* | 0-125m |
| *M4.2* | *Phytonet\_010* | 0-125m |
| *M4.2* | *CTD\_42 – Bottlenet* | 250-500m |
| *M4.2* | *CTD\_46 – Niskin bottle* | 10, 60, 125, 300m |
| *M4.2* | *CTD\_46 – Bottlenet* | 125-250m |
| *M4.2* | *CTD\_47 – Bottlenet* | 500-1000m |
| *M2.3* | *CTD\_49 – Niskin bottle* | 10, 60, 125, 300m |
| *M2.3* | *CTD\_49 – Bottlenet* | 125-375m |
| *M2.3* | *Phytonet\_011* | 0-125m |
| *M2.3* | *Phytonet\_012* | 0-125m |
| *M2.3* | *CTD\_53 – Bottlenet* | 125-375m |
| *M3.3* | *CTD\_57 – Bottlenet* | 200-1500m |
| *M3.3* | *Phytonet\_014* | 0-125m |
| *M3.3* | *CTD\_59 – Niskin bottle* | 10, 60, 125, 300m |

# INSTRUMENTS

Instrument Type: *47mm and 90mm filtration system with peristaltic pump for Niskin bottle samples; 25mm filtration system with aquarium pump for Phytonet and Bottlenet samples; Illumina MiSeq for paired-end-sequencing (2x300bp)*

Manufacturer:

Model:

Instrument Features / Calibration: **N/A**

# DESCRIPTION of PARAMETERS

## Measurement details

*Niskin bottle samples*

Seawater samples were collected with CTD cast at 4 depths (15m, 60m, 125m and 300m) at all visited stations. Before filtration, sampled seawater was prefiltered using a 100 μm mesh, in order to remove metazoa and large particles.

10L of water from each depth were successively filtered onto 20 µm and 0.2 µm to determine the taxonomic composition of large organisms (100 µm – 20 µm) and smaller organisms (20 µm - 0.2 µm). To describe the overall taxonomic diversity, half of the 20µm and 0,2µm filters at each depth was directly frozen and stored at -80°C for 18S rDNA metabarcoding. The other half was placed in RNAlater for one hour at room temperature before being stored at -80°C. It will be used to estimate the composition of the active community members through 18S rRNA sequencing.

Phytonet samples

Microplankton (>35 μm) was collected up to three times at each station with phytonet tows from 125 m depth to surface resulting in 14 phytonet samples. Due to the high visible abundance of copepods in the plankton net samples, the samples were pre-filtered through a 100 μm nylon mesh to remove the majority of the metazoans. Ten to 30 ml of concentrated phytonet sample were collected on 0.2 μm 25 mm diameter polycarbonate filters. These samples were preserved with RNAsafeguard Reagent and stored at -80°C for sequencing of the 18S rDNA to assess eukaryotic diversity.

Bottlenet samples

Bottlenets, to concentrate organisms >20 μm at specific depth, were employed during 2 to 4 CTD casts at each site resulting in 18 samples. The samples were prefiltered through 100 μm mesh and cells from 6 ml were collected on 0.2 μm 25 mm diameter polycarbonate filters. These samples were preserved with RNAsafeguard Reagent and stored at -80°C for sequencing of the 18S rDNA to assess eukaryotic diversity.

## Analytical procedure

In the laboratory, DNA and RNA will be extracted for 18S rRNA and rDNA sequencing. After DNA/RNA extraction, the diversity will be determined by high-throughput sequencing of the 18S rRNA genes on a Illumina MiSeq lane (paired-end 2x 300bp) (raw sequencing data: UC01-Amplicon-LOG\_R1\_001.fastq.gz, UC01-Amplicon-LOG\_R2\_001.fastq.gz).

## Units

N/A

## Sensor precision

N/A

## Post-cruise data analysis/treatment required

The sequences will be analyzed using various bioinformatic tools. Sequences will be demultiplexed and oriented with the Qiime1 pipeline. Filtering, trimming, clustering into ASVs (amplicon sequencing variants) and taxonomic assignments will be performed in R package DADA2 based on the database PR2 v.4.11.0. Statistical analyses of community composition and distribution across sampling sites will be conducted in the R packages phyloseq, microbiomeseq and vegan. For these analyses, a fasta file with sequences of all ASV (ASVs\_seq.fa), a table containing the taxonomic assignment of all ASV (ASVs\_taxonomy.txt), a table with number of reads per ASV in all samples (ASVs\_counts.txt), and a meta-data file containing sampling parameters of all samples (18Smetadata.txt) will be created.

## Estimated Date of Delivery

Summer 2019.

# BIBLIOGRAPHY

Christaki U., Georges C., Genitsaris S., Monchy S., 2015. Microzooplankton community associated with phytoplankton blooms in the naturally iron-fertilized Kerguelen area (Southern Ocean), *FEMS Microbiology Ecology*, **91**(7), fiv068.

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Georges C., Monchy S., Genitsaris S., Christaki U., 2014. Protist community composition during early phytoplankton blooms in the naturally iron-fertilized Kerguelen area (Southern Ocean). *Biogeosciences*, **11**, 5847-5863.
<https://doi.org/10.5194/bg-11-5847-2014>