Dataset name: **Parasites of microbial eukaryotes**

|  |  |
| --- | --- |
| Parameters: | * **Amplicon sequencing data from epicPCR (fastq file)**
* **Sanger sequencing data from single cell PCR (fasta file)**
* **Excel list with present parasite OTUs and infected host taxa**
* **FISH images**
* **Parasite & infected cell counts (excel file)**
 |

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

Address: **Mediterranean Institute of Oceanolography**

 **Institut Pytheas - Observatoire des Sciences de l'Univers**

 **Bâtiment OCEANOMED, Campus de Luminy, case 901**

 **F-13288 Marseille Cedex 09, France**

Chief scientist: **Ingrid Obernosterer** ingrid.obernosterer@obs-banyuls.fr

Address: **Laboratoire d’Océanographie Microbienne**

 **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: **Urania Christaki**

LOG

32 avenue Foch,

62930 Wimereux, France

+33 (0)3 21 99 64 01

Urania.christaki@univ-littoral.fr

Dataset contact: **Ingrid Sassenhagen**

LOG

32 avenue Foch,

62930 Wimereux, France

+33 (0)3 21 99 64 35

ingrid.sassenhagen@univ-littoral.fr

ingrid.sassenhagen@gmail.com

# OPERATIONS

## Sampling device(s)

Rosette bottlenet (usually through 125 m to surface) and bottles (15 and 60 m) from e–DNA casts and phytoplankton net (35 µm mesh) (vertical tows down to 200 m).

## List of stations sampled

**Table 1 : Sampled stations, sampling devices and type of analyses to be performed**

|  |  |  |
| --- | --- | --- |
| **Station** | **Sampling device and operation code** | **Type of analyses** |
| M2.1 | Phytonet\_001 | epicPCR, single cells |
| M2.1 | Phytonet\_002 | epicPCR |
| M2.1 | CTD\_007 - Bottlenet | FISH |
| M4.1 | Phytonet\_003 | epicPCR, single cells |
| M4.1 | Phytonet\_004 | epicPCR |
| M4.1 | CTD\_013 – Bottlenet | FISH |
| M4.1 | CTD\_014 – Bottlenet | FISH |
| M4.1 | CTD\_015 - Bottlenet | FISH |
| M3.1 | Phytonet\_005 | epicPCR, single cells |
| M3.1 | CTD\_21 – Bottlenet | FISH |
| M3.1 | Phytonet\_006 | epicPCR |
| M3.1 | CTD\_23 – Bottlenet | FISH |
| M3.1 | CTD\_25 - Bottlenet | FISH |
| M2.2 | CTD\_27 - Bottlenet | FISH |
| M2.2 | Phytonet\_007 | epicPCR, single cells |
| M2.2 | CTD\_29 – Bottlenet | FISH |
| M2.2 | CTD\_30 – Bottlenet | FISH |
| M1.1 | CTD\_35 – Bottlenet | FISH |
| M1.1 | CTD\_36 – Bottlenet | FISH |
| M1.1 | Phytonet\_008 | epicPCR, single cells |
| M1.1 | CTD\_037 – Bottlenet | FISH |
| M1.1 | CTD\_039 – 14, 22 | cell traps for epicPCR |

**Table 1 : Sampled stations, sampling devices and type of analyses to be performed (cont'd)**

|  |  |  |
| --- | --- | --- |
| **Station** | **Sampling device and operation code** | **Type of analyses** |
| M4.2 | Phytonet\_009 | epicPCR, single cells |
| M4.2 | Phytonet\_010 | epicPCR |
| M4.2 | CTD\_042 – Bottlenet | FISH |
| M4.2 | CTD\_044 – Bottlenet | FISH |
| M4.2 | CTD\_045 – Bottles 15 and 22 | Cell traps for epicPCR |
| M4.2 | CTD\_046 – Bottlenet | FISH |
| M4.2 | CTD\_047 – Bottlenet | FISH |
| M2.3 | CTD\_049 – Bottlenet | FISH |
| M2.3 | Phytonet\_011 | epicPCR |
| M2.3 | CTD\_050 – Bottles 15 and 22 | Cell traps for epicPCR |
| M2.3 | Phytonet\_012 | epicPCR, single cells |
| M2.3 | CTD\_053 – Bottlenet | FISH |
| M3.3 | CTD\_057 – Bottlenet | FISH |
| M3.3 | Phytonet\_014 | epicPCR, single cells |
| M3.3 | CTD\_060 – Bottles 9 and 20 | Cell traps for epicPCR |

# INSTRUMENTS

Instrument Type: **Inverted microscope**

Manufacturer: **Nikon**

Model: **Eclipse**

Instrument Features / Calibration: **100 – 400 x magnification**

# DESCRIPTION of PARAMETERS

## Measurement details

All samples for every analysis were prefiltered through 100 µm mesh to remove zooplankton.

Samples for epicPCR were taken from every phytonet and from the rosette deployment eDNA CTD casts (5 L from 15m and 60m each). 25 mL of Pluronic were added to the 5 L from the eDNA casts (final concentration 0.05 %). These samples were prefiltered through 20 µm to remove larger eukaryotic cells and concentrated to approximately 2 mL in cell traps. Glycerol was added to 10 mL phytonet samples and to 2mL cell trap samples to final concentrations of 25%.

6 mL from each prefiltered bottlenet sample were fixed with 150 µL formol (1% final), incubated for 1h at 4°C and filtered onto 25 mm, 5 µm pore size polycarbonate filters for FISH analyses with probes specific for MALV to identify infected phytoplankton cells. FISH samples from surface water collected for parameter "Total carbon uptake" (see cruise report) will be used for analyses of free MALV zoospores.

17 to 39 single phytoplankton cells were isolated with micropipetting from phytonet samples (see table 1 above) into 0.7 mL PCR tubes. Every individual cell was washed at least 3 times in 0.2 µm filtered seawater before final transfer into the tube. Ribosomal markers from the isolated phytoplankton cells and potential parasites will be sequenced for taxonomic identification using single cell PCR.

All the samples for the different analyses were stored at –80°C.

## Analytical procedure

To identify parasites, especially marine alveolates (MALV) (Epstein & Lopez-Garcia, 2008; Velo-Suárez *et al.*, 2013; Blanquart *et al.*, 2016), in phytoplankton cells, we will use epicPCR (Spencer *et al.*, 2016). Phytonet samples will be used to identify large (35–100 µm) protist cells infected by parasites, while the cell trap samples concentrated small protists (< 20µm).

EpicPCR separates microbial cells in environmental samples from each other in emulsion droplets. Phylogenetic markers from host and parasite will be amplified in the same droplet, anchored to each other using adaptors and sequenced in tandem (Spencer *et al.*, 2016). This method will be established with Manu Tamminen (University of Turku, Finland), who developed the technique, at ESE and LOG. Amplicon sequencing data from epicPCR will be analysed using pipelines in either Qime or Mothur.

Ribosomal markers (including ITS) will be amplified from single cells. These fragments will be cloned and sequenced using Sanger to phylogenetically identify both protist hosts and parasites.

We will develop FISH probes specific for ribosomal markers of MALV and utilize these to detect free zoospores and parasites in protist cells under the microscope following standard protocols (Not *et al.*, 2002).

## Units

N/A

## Sensor precision

N/A

## Post-cruise data analysis/treatment required

N/A

## Estimated Date of Delivery

* Amplicon sequencing data from epicPCR will be analysed by spring 2019.
* Phylogenetic analysis of single cell samples will be completed by spring 2019.
* Analysis of FISH filters will be done in summer 2019.

# BIBLIOGRAPHY

Blanquart F., Valero M., Alves-de-Souza C., Dia A., Lepelletier F., Bigeard E., Jeanthon C., Destombe C., Guillou L., 2016. Evidence for parasite-mediated selection during short-lasting toxic algal blooms. *Proceedings of the Royal Society B: Biological Sciences*, **283**, 20161870.

Epstein S., López-García P., 2008. “Missing” protists: a molecular prospective. *Biodiversity and Conservation*, **17**, 261–276.

Not F., Simon N., Biegala I.C., Vaulot D., 2002. Application of fluorescent in situ hybridization coupled with tyramide signal amplification (FISH TSA) to assess eukaryotic picoplankton composition. *Aquatic Microbial Ecology*, **28**, 157–166.

Spencer S.J., Tamminen M.V., Preheim S.P., Guo,M.T., Briggs, A.W., Brito I.L., Weitz D.A., Pitkänen L.K., Vigneault F., Virta M.P., Alm E.J., 2016. Massively parallel sequencing of single cells by epicPCR links functional genes with phylogenetic markers. *The ISME Journal*, **10**, 427–436.

Velo-Suárez L., Brosnahan M.L., Anderson D.M., McGillicuddy Jr. D.J., 2013. A quantitative assessment of the role of the parasite Amoebophrya in the termination of *Alexandrium* *fundyense* blooms within a small coastal embayment. *PLoS ONE*, **8**(12): e81150.