Dataset name: **Grazing of bacteria**

|  |  |
| --- | --- |
| Parameters: | * **Rates of bacterial consumption** |

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](mailto: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](mailto: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](mailto:Urania.christaki@univ-littoral.fr)

Dataset contact: **Urania Christaki**

LOG

32 avenue Foch,

62930 Wimereux, France

+33 (0)3 21 99 64 01

[Urania.christaki@univ-littoral.fr](mailto:Urania.christaki@univ-littoral.fr)

# OPERATIONS

## Sampling device(s)

Water was sampled from the rosette bottles at a single depth (30 m).

## List of stations sampled

M2\_1,M2\_2, M2\_3, M3\_1, M4\_1, M1 (CTD–eDNA)

# INSTRUMENTS

Instrument Type: **Flow cytometer CYTOFLEX epifluorescence microscope**

Manufacturer: **BECKMANN, Zeiss**

Model:

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

# DESCRIPTION of PARAMETERS

## Measurement details

Grazing of bacteria by small microbial eukaryotes is based on the use of fluorescently labelled bacteria (FLB) that are prey analogues. These are heat-killed bacteria stained with a fluorochrome (DTAF). Hence, they cannot divide and grazing rates can therefore be measured by following their disappearance over time. *Pseudomonas diminuta* (Vasquez-Dominquez *et al.,* 1999), the strain used to prepare the FLB, of 0.064 µm3 in size, is very similar to the natural bacteria.

Bottles of 1 L filled with 60 µm filtered seawater and 0.2 µm filtered water for the control were inoculated with approximately 0.5 x 105 ml-1 monodispersed FLB. Each treatment was run in duplicate. After the addition of the FLB, subsamples for microscopy and flow cytometry were immediately withdrawn for T0 counts of bacteria, FLB, and heterotrophic nanoflagellates (HNF). The three bottles were placed on a deck incubator with running surface seawater covered with a screen providing 75 % light attenuation. After 4h of incubation, 45 mL samples were taken in the bottles for inspection of FLB ingestion by HNF and estimation of their grazing rates (Dolan & Šimek, 1999). As a complementary approach, samples were taken at 3–4 more occasions during the 48–72 h of incubation for flow cytometry analyses of bacteria, FLB, HNF, and pigmented phytoplankton abundances during the experiment.

Bacterial grazing rates will be calculated by substracting the disappearance of FLB in the bottles containing the HNF from the FLB disappearance in the bottles without HNF. To complement the picture and for direct visualization of the organisms, samples for epifluorescence microscopy stained with DAPI were also taken for FLB and bacteria (10 mL) and nanoplankton (45 mL). All samples were kept frozen until analysis by flow cytometry and/or microscopy.

## Analytical procedure

Back in home lab, samples will be analysed with flow cytometry and/or epifluorescence microscopy.

## Units

Specific grazing rates (d–1) and community grazing rates (Bacteria ml–1 d–1)

## Sensor precision

N/A

## Post-cruise data analysis/treatment required

N/A

## Estimated Date of Delivery

Spring 2019.

# BIBLIOGRAPHY

Dolan J.R., Šimek K., 1999. Diel Periodicity in *Synechococcus* populations and grazing by heterotrophic nanoflagellates: Analysis of food vacuole contents. *Limnology & Oceanography*, **44**, 1565–1570.

Marie D., Partensky F., Vaulot D., Brussaard C., 1999. Enumeration of Phytoplankton, Bacteria, and Viruses in Marine Samples, in: Current Protocols in Cytometry, , Robinson J. (ed.), John Wiley and Sons Inc., New York., 1–15, 1999.

Vazquez-Dominguez E., Peters F., Gasol J.M., Vaqué D., 1999. Measuring the grazing losses of picoplankton: methodological improvements in the use of fluorescently labeled tracers combined with flow cytometry. *Aquatic Microbial Ecology*, **20**, 119–128.

Christaki U., Giannakourou A., Van Wambeke F., Grégori G., 2001. Nanoflagellate predation on auto- and heterotrophic picoplankton in the oligotrophic Mediterranean Sea. *Journal of Plankton Research*, **23**(11), 1297-1310.

Brussaard C., 2004. Optimization of procedures for counting viruses by flow cytometry. Applied and Environmental Microbiology, **70**, 1506–1513.

Christaki U., Courties C., Massana R., Catala P., Lebaron P., Gasol J.M., Zubkov M.V., 2011. Optimized routine flow cytometric enumeration of heterotrophic flagellates using SYBR Green I. *Limnology & Oceanography: Methods*, **9**, 329–339

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.   
 <https://doi.org/10.5194/bg-11-6739-2014>