Dataset name: **MAR–FISH (MicroAutoRadioactivity in combination with Fluorescence In Situ Hybridization)**

|  |  |
| --- | --- |
| Parameters: | * **Metabolic activity of bacteria and archaea assessed via assimilation of radiolabeled leucine** |

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**

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# OPERATIONS

## Sampling device(s)

Water samples were collected from the rosette bottles and *in situ* microbial incubator (ISMI).

## List of stations sampled

**Table 1: Details of sampled stations**

|  |  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| **Station** | **Date** | **Cast-ID** | **Depth** | **Bottle #** |  | **Station** | **Date** | **Cast-ID** | **Depth** | **Bottle #** |
| **M2\_1** | 26/02/18 | CTD-005 | 450 m | 1 |  | **M1** | 09/03/18 | CTD-305 | 200 m | 15 |
| **M2\_1** | 27/02/18 | ISP\_001 | 450 m | - |  | **M1** | 09/03/18 | CTD-305 | 500 m | 11 |
| **M4\_1** | 02/03/18 | CTD-017 | 100 m | 18 |  | **M1** | 09/03/18 | CTD-305 | 1000 m | 8 |
| **M4\_1** | 02/03/18 | CTD-017 | 200 m | 14 |  | **M1** | 09/03/18 | CTD-305 | 2000 m | 5 |
| **M4\_1** | 02/03/18 | CTD-017 | 500 m | 9 |  | **M1** | 09/03/18 | CTD-305 | 2500 m | 1 |
| **M4\_1** | 02/03/18 | CTD-017 | 1000 m | 6 |  | **M4\_2** | 14/03/18 | ISP\_006 | 3500 m | - |
| **M4\_1** | 02/03/18 | CTD-017 | 1900 m | 3 |  | **M2\_3** | 16/03/18 | CTD-052 | 50 m | 10 |
| **M4\_1** | 02/03/18 | CTD-018 | 3000 m | 21 |  | **M2\_3** | 16/03/18 | CTD-052 | 175 m | 14 |
| **M4\_1** | 02/03/18 | CTD-018 | 4000 m | 19 |  | **M2\_3** | 16/03/18 | CTD-052 | 300 m | 11 |
| **M4\_1** | 03/03/18 | ISP\_002 | 4370 m | - |  | **M2\_3** | 16/03/18 | CTD-052 | 350 m | 6 |
| **M3** | 04/03/18 | CTD-026 | 100 m | 19 |  | **M2\_3** | 16/03/18 | CTD-052 | 400 m | 4 |
| **M3** | 04/03/18 | CTD-026 | 200 m | 14 |  | **M2\_3** | 16/03/18 | CTD-052 | 500 m | 1 |
| **M3** | 04/03/18 | CTD-026 | 500 m | 10 |  | **M2\_3** | 17/03/18 | ISP\_007 | 175 m | - |
| **M3** | 04/03/18 | CTD-026 | 1000 m | 6 |  | **M3\_2** | 18/03/18 | CTD-057 | 100 m | 20 |
| **M3** | 04/03/18 | CTD-026 | 1500 m | 1 |  | **M3\_2** | 18/03/18 | CTD-057 | 200 m | 17 |
| **M3** | 04/03/18 | ISP\_003 | 1470 m | - |  | **M3\_2** | 18/03/18 | CTD-057 | 500 m | 13 |
| **M2\_2** | 06/03/18 | CTD-029 | 400 m | 4 |  | **M3\_2** | 18/03/18 | CTD-057 | 800 m | 10 |
| **M2\_2** | 06/03/18 | CTD-029 | 500 m | 1 |  | **M3\_2** | 18/03/18 | CTD-057 | 1000 m | 7 |
| **M2\_2** | 08/03/18 | ISP\_004 | 400 m | - |  | **M3\_2** | 18/03/18 | CTD-057 | 1500 m | 1 |
| **M1** | 09/03/18 | CTD-305 | 100 m | 18 |  | **M3\_3** | 19/03/18 | ISP\_008 | 1500 m | - |

# INSTRUMENTS

Instrument Type: ***In Situ* Microbial Incubator**

Manufacturer: **NIGK Corporation**

Model: **NWS-P6SC4K**

Instrument Features / Calibration: N/A

# DESCRIPTION of PARAMETERS

## Measurement details

The *in situ* microbial incubator, as the name suggests, is an instrument to incubate microorganisms at the depth of sampling and hence, without change of the hydrostatic pressure. During the cruise, prokaryotes were incubated *in situ* with the ISMI by lowering via winch from the research vessel.

Samples for fluorescence in situ hybridization combined with microautoradiography were incubated in plastic tubes (Greiner Bio-One). To volumes between 20 mL and 80 mL (depending on the depth) [3H]-leucine was added (5 nM final concentration), similar to the activity measurements. After 5 to 24 h the samples were fixed with 2% filtered formaldehyde and incubated for up to 18 h at 4ºC to fix the cells. Subsequently the samples were filtered onto 0.2-µm polycarbonate filters (25 mm diameter, Millipore GTTP) and rinsed with Milli–Q water. The filters were placed into 2 mL microfuge tubes and dried. Finally, the tubes with the filters were frozen at –80ºC until analysis in the home laboratory.

## Analytical procedure

CAtalyzed Reporter Deposition Fluorescence In Situ Hybridization (CARD-FISH) will be carried out as described in the metadata file:   
 MOBYDICK\_Metadata\_MOVIE\_MB\_Prokaryotic CARD-FISH.docx

In a darkroom, the hybridized filter sections will be transferred onto pre-cleaned microscopic glass slides coated with photographic emulsion (Ilford K.5; Ilford Photo) to a thickness of ~10 μm. The slides will be incubated at 4°C in a light-tight box containing silica beads for 6 to 8 d. After exposure, the slides will be developed and fixed according to the manufacturer’s specification (Ilford Photo). Slides will be examined at x 1250 magnification under an epifluorescence microscope and the images will be either manually or automatically evaluated.

## Units

* Leucine uptake amol cell–1 h–1

## Sensor precision

N/A

## Post-cruise data analysis/treatment required

N/A

## Estimated Date of Delivery

1 year after cruise end

# BIBLIOGRAPHY

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Herndl G.J., Reinthaler T., Teira E., van Aken H., Veth C., Pernthaler A., Pernthaler J., 2005. Contribution of Archaea to total prokaryotic production in the deep Atlantic Ocean. *Applied & Environmental Microbiology*, **71**, 2303-2309.

Sintes E., Herndl G.J., 2006. Quantifying substrate uptake by individual cells of marine bacterioplankton by catalyzed reporter deposition fluorescence *in situ* hybrid- ization combined with microautoradiography. *Applied and Environmental Microbiology*, **72**, 7022–7028.

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