Dataset name: **Prokaryotic CAtalyzed Reporter Deposition Fluorescence In Situ Hybridization (CARD–FISH)**

|  |  |
| --- | --- |
| Parameter: | * **Probe–targeted cell abundances (Archaea and Bacteria)**
 |

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 at every station

## List of stations sampled

**Table 1 : Sampling details**

|  |  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| **Sample ID #** | **Station** | **Cast #** | **Bottle #** | **Depth** |  | **Sample ID #** | **Station** | **Cast #** | **Bottle #** | **Depth** |
| 1A | M2\_1 | CTD\_005 | 22 | 10 m |  | 13B | M4\_1 | CTD\_017 | 9 | 500 m |
| 1B | M2\_1 | CTD\_005 | 22 | 10 m |  | 14A | M4\_1 | CTD\_017 | 6 | 1000 m |
| 2A | M2\_1 | CTD\_005 | 21 | 50 m |  | 14B | M4\_1 | CTD\_017 | 6 | 1000 m |
| 2B | M2\_1 | CTD\_005 | 21 | 50 m |  | 15A | M4\_1 | CTD\_017 | 3 | 1900 m |
| 3A | M2\_1 | CTD\_005 | 18 | 100 m |  | 15B | M4\_1 | CTD\_017 | 3 | 1900 m |
| 3B | M2\_1 | CTD\_005 | 18 | 100 m |  | 16A | M4\_1 | CTD\_018 | 21 | 3000 m |
| 4A | M2\_1 | CTD\_005 | 15 | 200 m |  | 16B | M4\_1 | CTD\_018 | 21 | 3000 m |
| 4B | M2\_1 | CTD\_005 | 15 | 200 m |  | 17A | M4\_1 | CTD\_018 | 19 | 4000 m |
| 5A | M2\_1 | CTD\_005 | 14 | 250 m |  | 17B | M4\_1 | CTD\_018 | 19 | 4000 m |
| 5B | M2\_1 | CTD\_005 | 14 | 250 m |  | 18A | M3\_1 | CTD\_026 | 21 | 50 m |
| 6A | M2\_1 | CTD\_005 | 13 | 350 m |  | 18B | M3\_1 | CTD\_026 | 21 | 50 m |
| 6B | M2\_1 | CTD\_005 | 13 | 350 m |  | 19A | M3\_1 | CTD\_026 | 18 | 100 m |
| 7A | M2\_1 | CTD\_005 | 1 | 450 m |  | 19B | M3\_1 | CTD\_026 | 18 | 100 m |
| 7B | M2\_1 | CTD\_005 | 1 | 450 m |  | 20A | M3\_1 | CTD\_026 | 15 | 200 m |
| 8A | M4\_1 | CTD\_017 | 21 | 25 m |  | 20B | M3\_1 | CTD\_026 | 15 | 200 m |
| 8B | M4\_1 | CTD\_017 | 21 | 25 m |  | 21A | M3\_1 | CTD\_025 | 1-22 | 350 m |
| 9A | M4\_1 | CTD\_017 | 18 | 100 m |  | 21B | M3\_1 | CTD\_025 | 1-22 | 350 m |
| 9B | M4\_1 | CTD\_017 | 18 | 100 m |  | 22A | M3\_1 | CTD\_026 | 10 | 500 m |
| 10A | M4\_1 | CTD\_014 | 12-22 | 150 m |  | 22B | M3\_1 | CTD\_026 | 10 | 500 m |
| 10B | M4\_1 | CTD\_014 | 12-22 | 150 m |  | 23A | M3\_1 | CTD\_026 | 6 | 1000 m |
| 11A | M4\_1 | CTD\_017 | 14 | 200 m |  | 23B | M3\_1 | CTD\_026 | 6 | 1000 m |
| 11B | M4\_1 | CTD\_017 | 14 | 200 m |  | 24A | M3\_1 | CTD\_026 | 1 | 1500 m |
| 12A | M4\_1 | CTD\_017 | 1-11 | 350 m |  | 24B | M3\_1 | CTD\_026 | 1 | 1500 m |
| 12B | M4\_1 | CTD\_017 | 1-11 | 350 m |  | 25A | M2\_2 | CTD\_029 | 22 | 15 m |
| 13A | M4\_1 | CTD\_017 | 9 | 500 m |  | 25B | M2\_2 | CTD\_029 | 22 | 15 m |

 **Table 1 : Sampling details (cont'd)**

|  |  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| 26A | M2\_2 | CTD\_029 | 21 | 25 m |  | 41B | M4\_2 | CTD\_041 | 1-12 | 350 m |
| 26B | M2\_2 | CTD\_029 | 21 | 25 m |  | 42A | M4\_2 | CTD\_044 | 11 | 2500 m |
| 27A | M2\_2 | CTD\_029 | 17 | 150 m |  | 42B | M4\_2 | CTD\_044 | 11 | 2500 m |
| 27B | M2\_2 | CTD\_029 | 17 | 150 m |  | 43A | M4\_2 | CTD\_044 | 7 | 3500 m |
| 28A | M2\_2 | CTD\_029 | 10 | 350 m |  | 43B | M4\_2 | CTD\_044 | 7 | 3500 m |
| 28B | M2\_2 | CTD\_029 | 10 | 350 m |  | 44A | M4\_2 | CTD\_044 | 2 | 4000 m |
| 29A | M2\_2 | CTD\_029 | 4 | 400 m |  | 44B | M4\_2 | CTD\_044 | 2 | 4000 m |
| 29B | M2\_2 | CTD\_029 | 4 | 400 m |  | 45A | M2\_3 | CTD\_052 | 20 | 25 m |
| 30A | M2\_2 | CTD\_029 | 1 | 500 m |  | 45B | M2\_3 | CTD\_052 | 20 | 25 m |
| 30B | M2\_2 | CTD\_029 | 1 | 500 m |  | 46A | M2\_3 | CTD\_052 | 14 | 175 m |
| 31A | M1 | CTD\_035 | 22 | 15 m |  | 46B | M2\_3 | CTD\_052 | 14 | 175 m |
| 31B | M1 | CTD\_035 | 22 | 15 m |  | 47A | M2\_3 | CTD\_052 | 11 | 300 m |
| 32A | M1 | CTD\_035 | 21 | 50 m |  | 47B | M2\_3 | CTD\_052 | 11 | 300 m |
| 32B | M1 | CTD\_035 | 21 | 50 m |  | 48A | M2\_3 | CTD\_052 | 6 | 350 m |
| 33A | M1 | CTD\_035 | 18 | 100 m |  | 48B | M2\_3 | CTD\_052 | 6 | 350 m |
| 33B | M1 | CTD\_035 | 18 | 100 m |  | 49A | M2\_3 | CTD\_052 | 4 | 400 m |
| 34A | M1 | CTD\_035 | 15 | 200 m |  | 49B | M2\_3 | CTD\_052 | 4 | 400 m |
| 34B | M1 | CTD\_035 | 15 | 200 m |  | 50A | M2\_3 | CTD\_052 | 1 | 500 m |
| 35A | M1 | CTD\_035 | 11 | 500 m |  | 50B | M2\_3 | CTD\_052 | 1 | 500 m |
| 35B | M1 | CTD\_035 | 11 | 500 m |  | 51A | M3\_3 | CTD\_057 | 20 | 100 m |
| 36A | M1 | CTD\_035 | 8 | 1000 m |  | 51B | M3\_3 | CTD\_057 | 20 | 100 m |
| 36B | M1 | CTD\_035 | 8 | 1000 m |  | 52A | M3\_3 | CTD\_057 | 17 | 200 m |
| 37A | M1 | CTD\_035 | 5 | 2000 m |  | 52B | M3\_3 | CTD\_057 | 17 | 200 m |
| 37B | M1 | CTD\_035 | 5 | 2000 m |  | 53A | M3\_3 | CTD\_057 | 13 | 500 m |
| 38A | M1 | CTD\_035 | 1 | 2500 m |  | 53B | M3\_3 | CTD\_057 | 13 | 500 m |
| 38B | M1 | CTD\_035 | 1 | 2500 m |  | 54A | M3\_3 | CTD\_057 | 10 | 800 m |
| 39A | M4\_2 | CTD\_044 | 22 | 50 m |  | 54B | M3\_3 | CTD\_057 | 10 | 800 m |
| 39B | M4\_2 | CTD\_044 | 22 | 50 m |  | 55A | M3\_3 | CTD\_057 | 7 | 1000 m |
| 40A | M4\_2 | CTD\_041 | 13-22 | 150 m |  | 55B | M3\_3 | CTD\_057 | 7 | 1000 m |
| 40B | M4\_2 | CTD\_041 | 13-22 | 150 m |  | 56A | M3\_3 | CTD\_057 | 1 | 1500 m |
| 41A | M4\_2 | CTD\_041 | 1-12 | 350 m |  | 56B | M3\_3 | CTD\_057 | 1 | 1500 m |

# INSTRUMENTS

Instrument Type: **Epifluorescence microscope**

Manufacturer: **Zeiss**

Model: **Axio Imager.M2**

Instrument Features / Calibration: N/A

# DESCRIPTION of PARAMETERS

## Measurement details

Seawater samples were collected from the rosette bottles into small polycarbonate bottles, each fixed with 37% Formaldehyde (2% final concentration) for 12–22 h at 4°C in the dark. Depending on the depth the sample volume was between 5 mL to 80 mL. After fixation samples were filtered onto 0.2 µm polycarbonate filters (25 mm diameter, GTTP, Millipore) and rinsed with Milli–Q water. Filters were dried and stored in a 2 mL tube, subsequently stored at –80°C until analysis in the home laboratory.

## Analytical procedure

CAtalyzed Reporter Deposition Fluorescence In Situ Hybridization (CARD-FISH) will be carried out as described previously (Teira *et al.*, 2006) with some modifications. Briefly, filters will be embedded in 0.1% low–gelling–point agarose and dried at 37°C for 10 min. Cell wall permeabilization will be achieved with a 1 minute 0.1 M HCl treatment for Archaea (Woebken *et al.,* 2007) and for Bacteria with a lysozyme permeabilization mix (10 mg mL–1 lysozyme, 0.1 M Tric-HCl, 0.05 M EDTA) at 37°C for 1 hour. Hybridization will be performed with horseradish peroxidase (HRP)–labeled oligonucleotide specific to target prokaryotic 16S rRNA gene sequences. For signal amplification filters will be incubated in a substrate mix with a tyramide solution with Alexa Fluor 488 and amplification buffer (10% dextran sulfate, 2 M NaCl, 0.1% blocking PBS) at 46°C for 15 minutes. After amplification, filters will be washed in PBS–T(0.05% Triton X100), Milli-Q water and dried before being mounted in a DAPI–PBS–Vectashield/Citifluor mix (DAPI 2 µg mL–1, 0.5 μg mL–1 PBS, 1 μg mL–1 Vectashield, 5.5 μg mL–1 Citifluor). Slides will be examined at x 1250 magnification under an epifluorescence microscope and the images will be either manually or automatically evaluated.

## Units

* Probe targeted cells cells mL–1

## Sensor precision

N/A

## Post-cruise data analysis/treatment required

N/A

## Estimated Date of Delivery

One year after cruise end for samples of interest (based on omics data).

# BIBLIOGRAPHY

Teira E., Lebaron P., van Aken H.M., Herndl G.J., 2006. Distribution and activity of Bacteria and Archaea in the deep water masses of the North Atlantic. *Limnology & Oceanography*, **51**, 2131–2144

Woebken D., Fuchs B.M., Kuypers M.M.M., Amann R., 2007. Potential interactions of particle-associated anammox bacteria with bacterial and archaeal partners in the Namibian upwelling system. *Applied & Environmental Microbiology*, **73**, 4648–4657.