FICHE META\_INFORMATION\_PARAMETRES

(à remplir par le responsable du paramètre)

### Nom du DATASET / Data SET NAME

*Bacterial abundance*

*Formazan*

*Bacterial respiration rate*

*Bacterial production*

*H2S determination*

### PROJET-ETUDE / *PROJECT TITLE*

*Campaign NAME*: AMOP *LEG: 1*

*Date* *begin: January 26th, 2014*

*Date end: February 22nd, 2014*

*Chief Scientist*: Aurélien PAULMIER, Boris DEWITTE, Véronique GARCON

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IFREMER -Centre de Brest

29280 PLOUZANE

### OPERATION *(if Relevant)*

*Station number-Cast number: 205, 302, 313, 317, 402, 1101, 1301, 1401, 1407, 1412, 1901, 2001, 2101, 2201, 2301, 2401, 2505, 2512, 2601, 2801, 2816.*

*Operation code:*

### RESPONSABLE SCIENTIFIQUE du paramètre / *PI of the parameter*

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| Nom /*name* | adresse / *address* | téléphone / *phone number* | fax /*fax number* | adresse mél /*email address* |
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### INFORMATION GEOGRAPHIQUES */ GEOGRAPHIC INFORMATION*

*Predefined site (if relevant):* Oxygen Minimum Zone (OMZ)

*Location:* Off Peru

*LATITUDE:* 7°50’S-14°34’S

*LONGITUDE:* 77°16’W-81°41’W

### DESCRIPTION DES INSTRUMENTS / INSTRUMENTS DESCRIPTION *(if Relevant)*

*Instrument Type: Epifluorescence microscope and a double beam spectrophotometer.*

*Manufacturer: Carl Zeiss, Perkin Elmer*

*Model:**Epifluorescence microscope (Axioskope II plus, Carl Zeiss, Oberkochen, Germany)*

 *Double beam spectrophotometer (Perkin Elmer Lambda 40, Waltham, MA, USA).*

*Instrument Features / Calibration: Plan-Apochromat, Carl Zeiss X100 objective; 175W xenon arc lamp; Lambda LS,Sutter connected through a liquid light guide with a triple Sedat filter, a dichroic filter with three transmission bands. Excitation and emission spectra were controlled by filter wheels (Lambda 10-3, Sutter). Images were captured with a cooled CCD camera (Clara E, Andor) with 10 ms integration time.*

*Formazan concentration was quantified based on calibration curves that were prepared using 1-(4-Iodophenyl)-5-(4-nitrophenyl)-3-phenylformazan from Sigma-Aldrich (cat. 17375).*

### DESCRIPTION DES PARAMETRES */ PARAMETERS DESCRIPTION*

# Ce qui a été collecté, mesuré et comment / *How was the parameter collected and measured (include references for analytical methods)?*

*Sampling: Niskin bottles-rosette. Sampling in the glass bottles at stations.*

*Analytical procedure : (briefly, could be a short recall to a published reference):*

***Bacterial abundance:*** *Twenty mL samples from Niskin bottles-rosette will be fixed with buffered formaldehyde (2 % final solution) and a 0.2 to 1 mL sample volume will be incubated with DAPI and filtered immediately on 0.2 µm black polycarbonate filters (Poretics). For each sample, a total of 10 fields were counted for a total of >300 cells using an* *epifluorescence microscope (Carl Zeiss) equipped with a X100 objective and a 175W xenon lamp (Lambda LS, Sutter) connected through a liquid light guide.*

***Formazan Measurement:****Niskin bottles-rosette. Sampling in a glass bottle at stations. A volume between 50 to 100 mL of each sample was incubated with INT (0.5 mmol/L final concentration) with incubation periods of less than 2 h. Samples were then filtered through 0.2 μm polycarbonate filters to collect the cells and the formazan crystals. These filters were immediately preserved (-20 Celsius, <2 days) or immediately extracted with 1.5 mL propanol with a homogenizer (Beadbeater, Cole-Parmer, Vernon Hills, IL, USA, 600 seconds at 5000 rpm). Blanks were prepared by killing samples with a 2% final solution of formaldehyde about 1 h before INT addition. The blanks were subtracted from the sample value. Formazan*

*concentration was measured at 485 nm.*

***Bacterial respiration rate:*** *Our method is based on the concept that the formazan produced represents the potential cell capacity to reduce INT in the prokaryote cells membrane, and is responsible for reducing the INT ontime scales of 1 h. We propose that the potential cell capacity to reduce INT can be used as a proxy for the respiration rate (Equation (1)).*

*1. R=0.20F2.15 , r2=0.93; p≤0.05*

*After 1 h the great majority of the total formazan had been produced. We explicitly suggest to use this method only for the estimation of prokaryotes, because the internal cell organization of eukaryotes complicates the interpretation of INT reduction results.*

***Bacterial production:*** *Bacterial production measurements in field samples were carried out following the method of Smith and Azam (1992) and incubated at surface water temperature. Briefly, triplicate 1.5 ml subsamples were incubated during 2.5 hours in the dark. The samples were counted on a PerkinElmer Tri-Carb 2810 TR scintillation counter.*

***Determination of hydrogen sulphide:*** *We used spectrophotometric determination of hydrogen sulphide. The determination of hydrogen sulphide as methylene blue (3,7-bis(dimethylamino)phenothiazine-5-onium chloride. The reagents, 0.5 mL of dimethyl-p-phenylenediamine and 0.5 mL iron chloride solution, were added to the sample with piston pipettes. No air bubbles must be trapped in the bottles. The colour develops within a few minutes). A spectrophotometer was used for absorbance readings to 670 nm.*

*Units: Bacterial Abundance [cell/mL], Formazan [μmol F/L], Bacterial Respiration rate [μmol O2 /m3/day], Bacterial Production [µg C /L/day], H2S [μmol/mL].*

*Sensor Precision:* For each sample, a total of 10 fields were counted for a total of >300 cells (Kirchman, 1993).

### REFERENCES BIBLIOGRAPHIQUES

1. Villegas-Mendoza J, Cajal-Medrano R, Maske H (2019) The Chemical Transformation of the Cellular Toxin INT (2-(4-Iodophenyl)-3-(4-Nitrophenyl)-5-(Phenyl) Tetrazolium Chloride) as an Indicator of Prior Respiratory Activity in Aquatic Bacteria. Int J Mol Sci 20:782.

2. Kirchman, D. Statistical analysis of direct counts of microbial abundance. In Handbook of methods in aquatic microbial ecology; Kemp, P.F., Sherr, B.F., Sherr, E.B., Cole, J.J., Eds.; Boca Raton, FL (1993) pp. 117–119 ISBN 1351442368.

3. Smith D.C. and Azam F. (1992). A simple, economical method for measuring bacterial protein synthesis rates in seawater using 3H-leucine. Marine Microbial Food Webs 6: 107-114.

4. Grasshoff K, Ehrhardt M, Kremling K (Eds.) (1999) Methods of Seawater Analysis; Verlag Chemie,Weinheim.

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*Estimated Date of Delivery :*