

Sampling protocols and measurements used during the September 2015 AMOR-BFlux Cruise

Jens Rassmann^{a,1}, Eryn M. Eitel^{b,1}, Bruno Lansard^a, Cécile Cathalot^c
Christophe Brandily^c, Martial Taillefert^b, Christophe Rabouille^{a, 2}

^a *Laboratoire des Sciences du Climat et de l'Environnement, LSCE/IPSL, CEA-CNRS-UVSQ-
Université Paris Saclay, 91198 Gif-sur-Yvette, France*

^b *School of Earth and Atmospheric Sciences; Georgia Institute of Technology, GA 30332-
0340 Atlanta, USA*

^c *IFREMER, Laboratoire Environnement Profond, 29280 Plouzané, France*

1. Bottom water sampling and analyses

The AMOR-B-Flux cruise took place on-board the RV Tethys II (CNRS-INSU) in September 2015. The investigated stations were located in the river plume along a nearshore-offshore transect (Fig. 1 and Table 2). Bottom water samples were collected with 12-L Niskin[®] bottles from 1-2 m above the sea floor. The sampling depth was checked with a mounted underwater depth gauge. The seawater temperature was measured using a thermometer with a precision of 0.1 °C and the salinity with a conductivity based thermosalinometer with a precision of 0.1. Dissolved oxygen concentrations were analysed by Winkler titration (Grasshoff et al., 1983) within twelve hours after sampling with a precision of $\pm 0.5 \mu\text{M}$. Triplicate pH measurements were carried out at 25°C within 1 hour after sampling by spectrophotometry with unpurified m-cresol purple as indicator dye (Clayton and Byrne, 1993) and a precision of ± 0.01 pH units. The CO2SYS software (Pierrot et al., 2006, Orr et al., 2018) was used with the equilibrium constants from Luecker et al. (2000) and DIC and pH as input parameters to report pH on the total proton scale (pH_T) and at *in situ* temperature and salinity. As silicate and phosphate concentrations were not measured in the bottom waters, average concentrations for the Gulf of Lions were used. To obtain concentrations as close as possible to the seafloor, overlying water from the sediment cores was also sampled and analysed for TA and DIC concentrations.

2. In situ benthic chamber deployments

Benthic fluxes were determined with an autonomous benthic lander (Jahnke and Christiansen, 1989). The lander was equipped with a single benthic chamber and water syringe sampling system. The chamber encloses a 30 x 30 cm sediment surface area with a volume of overlying

water determined by measuring the initial concentration of two tracers (iodide and bromide) injected after closure of the chamber within 20 minutes after deployment on the seafloor, to ensure that the chamber and particles eventually resuspended were settled in the sediment. A mechanical stirrer integrated in the chamber lid was run at 10 rpm to homogenize the overlying waters in the chamber without interfering with sediment-water exchange processes (Buchholtz-Ten Brink et al., 1989). TA and DIC samples were collected as a function of time and their concentrations corrected for the dilution that occurred by replacing the sample volume collected by ambient water. The slopes of the concentration-time plots were estimated using the “lm” function in R, a restricted maximum likelihood estimator (REML) that takes uncertainties of individual measurements into account. Finally, benthic fluxes across the SWI (F_i in $\text{mmol m}^{-2} \text{d}^{-1}$) were calculated from the slopes of these concentration-time-plots and the chamber height (Eq. 1),

$$F_i = H \cdot \frac{dC_i}{dt} \quad (1)$$

where H (m) is the overlying water height in the benthic chamber, C_i represents the concentration of the analyte i (TA or DIC, in mmol m^{-3}), and t is time (d).

3. In situ microprofiling of dissolved oxygen and pH

A separate benthic lander, carrying a benthic microprofiler (Unisense[®]), was deployed to measure *in situ* microprofiles of dissolved oxygen and pH (Cai and Reimers, 1993; Rabouille et al., 2003, Rassmann et al., 2016 and references therein). Up to five oxygen and two pH microelectrodes were simultaneously deployed, and vertical depth profiles were measured with a 200 μm resolution. As their response to variations in oxygen concentrations is linear, the O_2 microelectrodes (Boudreau and Jorgensen, 2001) were calibrated with a two-point calibration technique using the bottom water O_2 concentration determined by Winkler titration and the anoxic pore waters. The pH microelectrodes were calibrated using NBS buffers (pH 4.00, 7.00, and 9.00 at 20°C) and the spectrophotometrically determined pH of the bottom waters was used to correct for the difference in the liquid junction potential between seawater and the NBS buffers. Signal drift of O_2 and pH microelectrodes during profiling was checked to be less than 5 %.

4. Sediment sampling, porosity measurements, and ex situ voltammetric profiling

At each sampling station, six sediment cores (2 for pore waters, 1 for porosity, 1 for voltammetry, 1 for methane, and 1 for archives) were collected using an UWITEC[®] single corer (length 60 cm, inner diameter 9 cm) within 30 m from the site where the landers were deployed and processed within 30 minutes after collection. Sediment porosity profiles were determined by slicing one of the cores with a 2 mm resolution until 10 mm depth, a 5 mm resolution until 60 mm, and a 10 mm resolution down to the bottom of the cores. Porosity was calculated from the bottom water salinity, an average sediment density of 2.5 g cm^{-3} , and the weight difference between the wet and dried sediment after one week at $60 \text{ }^\circ\text{C}$.

Ex situ voltammetric profiles were obtained in a separate core with a AIS, Inc. DLK-70 potentiostat in a three electrode configuration, including Hg/Au working microelectrode constructed from Pyrex glass pulled to a tip of 0.4 mm diameter to minimize particle entrainment during the profiles (Luther et al., 2008), an Ag/AgCl reference electrode, and a platinum counter electrode. The Hg/Au voltammetric electrode was deployed in the sediment using a DLK MAN-1 micromanipulator (AIS, Inc). Using a combination of linear sweep and anodic and cathodic square wave voltammetry, Hg/Au voltammetric microelectrodes are able to simultaneously quantify dissolved O_2 , Mn^{2+} , Fe^{2+} , total dissolved sulfide (including elemental sulfur and polysulfides which cannot be easily distinguished from reduced sulphide; $\Sigma\text{H}_2\text{S} = \text{H}_2\text{S} + \text{HS}^- + \text{S}^0 + \text{S}_x^{2-}$), as well as organic complexes of Fe(III) (org-Fe(III)) and iron sulfide clusters (FeS_{aq}), which are not quantifiable but reported in normalized current intensities (Tercier-Waeber and Taillefert, 2008). Hg/Au microelectrodes were calibrated for dissolved O_2 using *in situ* temperature and salinity of the overlying waters to determine the dissolved O_2 concentrations at saturation (Luther et al., 2008). They were also calibrated externally with MnCl_2 to quantify all other species according to the pilot ion method (Luther et al., 2008). All voltammetric data was integrated using VOLTINT, a semi-automated Matlab[®] script with peak recognition software (Bristow and Taillefert, 2008).

5. Pore water and solid phase extractions and analyses

Between 12 and 15 ml of sediment pore waters at each sampled depth were extracted from a third sediment core using rhizon filters with a mean pore size of $0.1 \text{ } \mu\text{m}$ (Seeberg-

Elverfeldt et al., 2005) in a glove bag that was extensively flushed with N₂ to create an anaerobic atmosphere (monitored with an O₂ sensor and verified with a 2 % ((NH₄)₂Fe(SO₄)₂ · 6H₂O) solution). Pore waters were subsampled within 2 hours onboard. A sample volume of 1 ml was immediately used for dissolved phosphate analysis using the paramolybdate method (Murphy and Riley, 1962) as well as for dissolved Fe²⁺ and total dissolved iron analysis using the ferrozine method (Stookey, 1970). Pore water and bottom water fractions were poisoned with HgCl₂ for TA and DIC or acidified to pH 1 with ultrapure HCl for sulfate and stored at 4 °C until analysis in the laboratory. Total alkalinity was measured on 3 - 6 ml sample volume by open cell titration with 0.01 M HCl (Dickson et al., 2007). Depending on the available sample volume, duplicate or triplicate titrations were performed. DIC concentrations were analyzed with a DIC analyzer (Apollo/SciTech[®]) on 1 ml samples as previously described (Rassmann et al., 2016) and reported as the average and standard deviations of triplicate measurements. The TA and DIC methods were calibrated using certified reference materials for oceanic CO₂ measurements provided by the Scripps Institution of Oceanography (batch n°136). The relative uncertainty for both DIC and TA was ± 0.5 % of the final value. Sulfate concentrations were quantified on 100 µl sample volumes after dilution by ion chromatography on an ICS 1000 chromatograph (Dionex) with an IonPac AS 9 HC column and AG 9 HC guard by suppressed conductivity with an AERS 500 suppressor (ThermoFisher Scientific). A 9 mM solution of Na₂CO₃, at a flow rate of 1 ml min⁻¹ was used as the eluent. The relative uncertainty of this method was ± 1.6 %. To validate a newly developed high performance liquid chromatography method (Beckler et al., 2014), pore water fractions from a separate core were also frozen at -18 °C for sulfate analysis back in the laboratory. For this new method, a Waters, Inc. 1525 binary pump with Waters 2487 absorbance detector at 215 nm was used with a Metrohm Metrosep A Supp 5 anion exchange column (150 mm x 4.0 mm) with a 1.0 mM NaHCO₃ / 3.2 mM Na₂CO₃ eluent at a flow rate of 0.7 ml min⁻¹ (Beckler et al., 2014). To measure ammonium (NH₄⁺) concentrations, 1 ml subsamples were diluted and analysed using the indophenol blue method (Grasshof et al., 1983). The uncertainty of the method was about 5 %. Pore water fractions of 1 ml volume were also acidified with 2 % HCl for Ca²⁺ analysis by inductively-coupled plasma atomic emission spectroscopy (Ultima 2, Horiba Scientific). The method was validated with mono-elemental standards and standard solutions (IAPSO, CASS-4, and NASS-6 seawater reference materials) and displayed an external relative uncertainty of ± 2-3 % depending on the sample series and a detection limit of 100 µmol l⁻¹.

Close to the Rhône River mouth, at station A, Z, and AK, one additional core was subsampled for methane analysis with 1 cm diameter corers made of cut 10-ml syringes inserted every 5 cm through pre-drilled holes on the side of the core. Due to the technical challenge of sampling non soluble methane in pore waters, this sampling could not be carried out on the same cores as the other pore water analyses. The content of these subsamples was carefully inserted in gas tight vials containing deionized water and HgCl₂ solution and kept at 4°C until methane analysis. Dissolved methane was quantified after degassing of the pore waters into the headspace and quantified by gas chromatography with a relative uncertainty of ± 5 % (Sarradin and Caprais, 1996). The position of the sulfate-methane transition zone (SMTZ) was determined as the zone around the depth where [SO₄²⁻] = [CH₄] (Komada et al., 2016). Finally, acid volatile sulfur (AVS) for the determination of FeS_s was extracted from the same sediment used for the pore water extractions after slicing into layers of 1 to 5 cm. FeS_s measurements were conducted in triplicate by cold acid distillation of H₂S (g) under anoxic conditions that was trapped by NaOH and quantified voltammetrically (Henneke et al., 1991).

6. References

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