

Meta Data for CAR 180, 186, 191 and 201
submitted by MI Scranton and GT Taylor

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Methods: Contact Mary Scranton (mary.scranton@stonybrook.edu) for more details on any of the methods or the data.

Sampling: All samples are collected in standard 8 or 12-L Niskin bottles. For samples in and below the oxycline, an Argon line is attached to the upper air vent to prevent air from entering the bottle during sub-sampling. Samples for live analysis are first transferred without headspace to a 1L glass sample bottle with Teflon standard taper stopper. In the ship's lab, sub-samples are transferred to 25 or 40 ml incubation vials, under nitrogen. All vials are filled from the bottom with overflow of about 3 vial volumes and then sealed with no headspace.

CH₄: CH₄ is assayed by gas chromatography using the vial equilibration technique of Johnson et al. (1990) and an HP 5890IIA GC. The GC was calibrated for each run using three standards, containing 9.98, 999.3 and 2000 ppmv CH₄ in nitrogen (for CAR 180) and 5.10, 10.0 and 999.3 ppmv CH₄ in nitrogen for CAR 186, 191 and 201. Samples are poisoned by addition of 10N KOH solution at a rate of 250 microliter per 50 ml vial.

H₂S: Seawater samples for sulfide were collected without bubbles by placing the tip of a gas-tight syringe below the surface of water flowing upward through a 60 ml plastic syringe barrel which had been attached to the Niskin bottle by a 60 cm length of Tygon tubing. Samples are injected into vials containing 0.5 ml Zn-acetate (50 mM). Samples were chilled on the ship and stored refrigerated in the dark until analysis. Upon return to the laboratory, the ZnS is dissolved and is analyzed spectrophotometrically by the method of Cline (1969). Concentrations of sulfide in the standards was confirmed by back titration using a Winkler method to confirm the amount of water in the weighed reagent (Li and Astor, 2011).

Concentrations were calculated assuming a linear fit of the plot of concentration vs absorbance, although in fact the line is slightly curved. This results in slight overestimates of sulfide concentration near the detection limit and at very high concentrations but differences with polynomial fit are likely within the measurement error. Consult with Scranton for more details or raw data.

Sulfide samples from both our geochemistry cruise and the CARIACO time series cruise are reported for CAR 180. For CAR 186, Stony Brook sulfide samples from were lost during shipment due to leakage (old caps were used and the plastic may have become rigid.) For CAR 191 and 201 sulfide samples were collected during the biogeochemistry cruise and were analyzed at EDIMAR to avoid problems in shipping.

Sulfite and thiosulfate: Sulfite and thiosulfate were determined for CAR 180, 186 and 191. Following CAR191 we discontinued analysis of these species.

Seawater samples for thiosulfate and sulfite analyses were collected as for sulfide in triplicate without bubbles by placing the tip of a gas-tight syringe below the surface of water flowing upward through a 60 ml plastic syringe barrel which had been attached to the Niskin bottle by a 60 cm length of Tygon tubing. Samples were analyzed using the method of Vairavamurthy and Mopper (1990) as modified by Hayes et al. (2006). Ten-milliliter water samples were collected from Niskin bottles as described above and were transferred within seconds into a glass serum vial containing 0.5 ml sodium acetate buffer (0.2 M). All reaction vials were prepared in advance at the shore-based laboratory by adding buffer, flushing with argon and crimp sealing for transport to the field. To minimize oxidation, the derivatizing agent (5 mM 2,2'-dithiobis(5-nitro) pyridine in acetonitrile) was added within seconds of dispensing seawater into serum vials. Derivatization was allowed to proceed for 5 min, after which water was passed through preconditioned Waters SepPak tC18 Solid Phase Extraction (SPE) cartridges. Cartridges were preconditioned immediately before use with 5 ml methanol, 5 ml distilled water, and 5 ml of a mixture of 20 mM sodium acetate and 10 mM tetrabutylammonium hydrogen sulfate (TBAHS). Samples on cartridges were kept in a cooler on deck until the cast was completed and then were frozen. Upon returning to the local laboratory, cartridges were thawed, purged with argon and refrozen until analysis. Frozen samples are typically thawed for about 10 minutes prior to elution.

Upon return of the samples to Stony Brook, thiosulfate and sulfite derivatives were eluted from cartridges with methanol and analyzed on a Shimadzu HPLC consisting of a SCL 10A-VP system controller, two LC-10AT pumps, an SPD-10AV/VP ultraviolet detector, and a SIL-10A auto-injector. Mobile phases for analysis were (A) 100% acetonitrile and (B) a solution of 0.05 M sodium acetate and 7.5 mM TBAHS adjusted to pH 3.5 ± 0.03 . The gradient for this method was 1 min with 10% B followed by a gradient to 34% B at 9 min, to 55% B at 23 min, to 100% B at 28 min, continued elution with 100% B for 2 min, then a gradient back to 10% B at 32 min and to 0% B at 40 min. Absorbance of the derivatives was measured at 320 nm.

Lab blanks (10 ml of distilled water, buffer and 0.05 ml of the derivatizing agent (DTNP)) were concentrated through preconditioned SepPak cartridges and analyzed in the same manner as field samples and were 0.07 ± 0.04 micromolar for sulfite and 0.3 ± 0.1 micromolar for thiosulfate. The analytical detection limit (6x the standard deviation of five laboratory blanks) was 0.3 micromolar for sulfite and 0.6 micromolar for thiosulfate. Field blanks were assumed to be lower than the lowest measured sample in a given cast. Upper estimates of the true blanks are the lowest thiosulfate and sulfite value which were measured during a particular cruise were 0.3 micromolar and 0.5 micromolar, respectively. The precision of analysis (relative standard deviation of 5 replicates of a 10 micromolar standard) for thiosulfate and sulfite was $\pm 2.2\%$ and $\pm 1.6\%$ respectively.

Elemental sulfur: Duplicate particulate elemental sulfur samples were acquired by gravity filtering directly from the Niskin bottles as described by Trouwborst (2005) and were analyzed by a modification of the method of Henneke et al. (1997). Filter holders, loaded with 0.2 μm polycarbonate filters, were attached to the Niskin bottle by Tygon® tubing. Filtrate was collected for each filter in a graduated cylinder to determine the filtered volume. The filters were dried

by passing argon gas through the filters and stored in 15 ml centrifuge tubes at -20 °C. After return to Stony Brook University, 6 ml methanol was added to each centrifuge tube to extract elemental sulfur from the filter. The centrifuge tubes were shaken for 2.5 hours on a mechanical shaker and the S^0 concentration of each sample was analyzed on a Shimadzu HPLC consisting of a SCL 10A-VP system controller, two LC-10AT pumps, an SPD-10AV/VP ultraviolet detector, and a SIL-10A auto-injector. We used a ODS hypersil C_{18} reverse phase, 250 mm \times 4.6 mm, 5 μ m column (Supelco Co.) at room temperature. Twenty μ l samples were injected into the chromatograph and eluted with 98% methanol/2% water at a pump speed of 1 ml/min. Retention time of the elemental sulfur peak was typically about 2.2 min. Elemental sulfur was detected at 226 nm, with a detection limit of about 1 μ mol L^{-1} , and a precision of 0.5% relative standard deviation among replicates. Standard solutions, made by dissolving sulfur powder in methanol and serially diluting, are linear in the range of 1-100 μ mol L^{-1} .

Total Zero-valent Sulfur:

Samples (40 ml) were obtained with a 60 ml plastic syringe from flowing seawater as described for the thiosulfate and sulfite samples. The sample then was added to a 50 ml centrifuge tube containing 2 ml of 2% (w/v) Zn-acetate. Samples were well shaken and then frozen. On return to Stony Brook, samples were warmed to room temperature. One ml chloroform was added to each tube to extract elemental sulfur and the tube was vortexed for 1 min. Then the tube was allowed to sit for 10 mins. The chloroform is denser than the seawater, so the chloroform will be at the bottom and there is an obvious layer differentiation between chloroform and seawater. Using a Pasteur pipette, the chloroform layer was transferred to a 1.5 ml HPLC vial. The extraction was then repeated a second time with another 1 ml chloroform which is added to the same HPLC vial. This increases extraction efficiency to >90%. The pooled extraction was diluted 1:3 with methanol to optimize chromatography. The HPLC mobile phase was the same as that used for elemental S, but the flow rate was reduced to 0.5 ml/min. The retention time of total zero valent sulfur was around 5.5 minutes.

Total zero-valent sulfur data for CAR 180 seem questionable as values were high and relatively constant across the oxic-anoxic interface. We have no explanation for this but have included the data here.

Microbial census: Abundances of remineralizers (bacteria) and regenerators (flagellates) are determined using microscopic censuses. Preserved samples (2% formaldehyde) are stained with a fluorochrome (DAPI or acridine orange) and captured on the appropriate porosity Nuclepore membrane (0.2 or 0.8 μ m). Filter-retained cells are enumerated and sized by epifluorescence microscopy according to Taylor et al. (1986). Larger, less abundant protozoa are enumerated on settled samples using inverted microscopy.

Bacterial production: Bacterial incorporation is measured using 3H -leucine incorporation as described by Kirchman (1993). Triplicate samples are incubated for 10-12 h in gas-tight screw-top vials to minimized alteration of the redox potential. Time course experiments have confirmed that uptake is linear for at least 15 h. Due to the fact that some important anaerobic bacteria appear to not take up exogenous thymidine under anoxic conditions (McDonough et al. 1986; Gilmour et al. 1990), the more common method of Fuhrman and Azam (1982) is inappropriate for this system.

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