Data for CAR 153 and CAR 157 submitted by MI Scranton and GT Taylor
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Marine Sciences Research Center
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Methods:

Sampling: All samples are collected in standard 8 or 12-L Niskin bottles. For samples in and below the oxycline, an N2 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, also under N2. All vials are filled from the bottom with overflow of about 3 vial volumes and then sealed with no headspace.

Fatty acid uptake rate constants: Fatty acid measurements have been discontinued. Contact Mary Scranton for further information.

CH4: CH4 is assayed by gas chromatography using the vial equilibration technique of Johnson et al. (1990) and an HP 5890GC. The GC was calibrated for each run using two standards, one containing 9.98 ppmv CH4 in nitrogen and the other containing 999.3 ppmv CH4 in nitrogen. The two point calibration yields a slightly higher concentration (by about 15%) for deep water values, than data reported prior to CAR 108. For more information contact Scranton. Samples are poisoned by addition of 10N KOH solution at a rate of 200 l per 50 ml vial.

For the data for CAR 153 and CAR 157 (January and May 2009) values of methane seem lower than we expected. We noted at the time for CAR 153 that the silicone sealant did not cure correctly and we strongly suspect that methane was lost from vial headspace as vials were outside in the heat for an extended period while a hole was in the septum and sealant was not cured. Thus we are not reporting data for CAR 153. For CAR 157 our notes are that the sealant cured normally, but values were still considerably lower than we had seen in CAR 145 and saw again in CAR 163. Thus we are reporting the data, although we have no explanation for the lower values. Contact Mary Scranton (mscranton@notes.cc.sunysb.edu) for more details.

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 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). Based on the relative standard deviation of triplicate samples, the precision of the field samples during analysis was ± 3.3%. The detection limit for sulfide analyses was 0.59 micromolar, calculated as six times the standard deviation of quintuplicate blanks. While standard curves appeared linear over a large concentration range as determined by a high correlation coefficient (greater than 0.98), close inspection showed they were consistently convex over the entire concentration range (from 0 to ~50 micromolar). Therefore, we matched concentrations of

standards to those in samples and kept total absorbance low to optimize analysis for the concentration range expected in the Cariaco Basin. We used a linear calibration equation to be consistent with previous sulfide measurements. We did not subtract a reagent blank from the samples. In some cases, this has lead to small positive values (~1 micromolar) in the suboxic zone.

Sulfite and thiosulfate: 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. Upon returning to the local laboratory, cartridges were purged with argon and frozen 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 much higher and are 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 and are 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 ± 2.2% and ± 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 rinsed with de-ionized water, 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 × 4.6 mm, 5 µm column (Supelco Co.) at room temperature. Twenty µl samples were injected into the chromatograph and eluted with 100% methanol 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 µmol 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 µmol L^{-1}.

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 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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