Meta Data for CAR 207, 212 and 216
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 5.10, 10.0 and 999.3 ppmv CH4 in nitrogen. 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 were 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.

For the present cruises, sulfide samples were collected during the biogeochemistry cruise and were analyzed at EDIMAR to avoid problems in shipping.

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 × 4.6 mm, 5 µm column (Supelco Co.) at room temperature. Twenty µl 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 2.5 min. Elemental sulfur was detected at 264 nm. Standard solutions, made by dissolving sulfur powder in methanol and serially diluting, are linear in the range of 1–100 µmol $L^{-1}$.

Total Zero-valent Sulfur:
Samples (40 ml) were obtained in duplicate with a 60 ml plastic syringe from flowing seawater sulfide without bubbles by placing the tip of a 60 ml plastic 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. 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 minutes. 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. (The chloroform does not separate completely, so the total volume of the two extractions is less than 1.5 ml) This increases extraction efficiency to >90%. The HPLC mobile phase was 90% methanol, 10% water, and the flow rate was reduced to 0.5 ml/min. The retention time of total zero valent sulfur was around 8.5 minutes. Standards were made up in chloroform rather than methanol as for the particulate sulfur to match the sample matrix.

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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