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CARIACO Ocean Time Series (Biogeochemistry component)
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Ship: For all cruises, the B/O Hermano Ginés, operated by the Fundación La Salle, Edimar, Venezuela

The goal of this project was to examine the interrelationship between microbial activity and water column geochemistry in the world's largest, truly marine anoxic system, the Cariaco Basin.

Sampling:
(CAR 1, 5, 9, 13, 19, 25, 29, 32, 36, 42, 48, 54, 60, 66, 74, 78, 89, 96, 100, 108, 108B, 108C, 112, 112B, 112C, 118, 118B 118C, 122, 122B, 122D, 128, 128B, 132, 132B, 132D, 139, 145, 153, 157, 163, 169, 175, 180, 186, 191, 201, 207, 212, 216, 224)
All samples were collected in standard 8 or 12-L Niskin bottles. For samples in and below the oxycline, a heavy wall Tygon tubing line was attached to the upper air vent to carry nitrogen or argon to the top of the Niskin to prevent air from entering the bottle during sub-sampling.

Depth indicates intended sample depth. ***Corrected depth*** indicates depth sample was actually taken from based on CTD record.

For geochemistry samples, water was collected into vials or containers as described under the individual analyses below. Samples for microbial census (bacteria and flagellates) were collected in 250 ml plastic bottles containing 25 ml of buffered formalin (final concentration 2%). Samples for live analysis (bacterial net production and dark carbon fixation, also called chemoautotrophy) were 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. For chemoautotrophy ground glass stoppered vials were used. For bacterial net production, glass vials equipped with a Teflon-lined septum and a screwcap were used. All vials are filled from the bottom with overflow of about 3 vial volumes and then sealed with no headspace.

Most cruises occupied only the CARIACO time series station. On cruises 108 to 132, additional stations in the western basin and on the sill to the north of the Cariaco station were sampled. Locations are given in the database.

Oxygen
(CAR 29, 32, 48, 54, 60, 66, 74, 78, 89, 96, 100, 108, 108B, 108C, 112, 112B, 112C, 118, 118B, 118C, 122, 122B, 122D, 128, 128B, 132, 132B, 132D, 139, 145, 153, 157, 163, 169, 175, 180, 186, 191, 201, 207, 212, 216, 224)
Oxygen concentrations presented in this table were determined using a modification of the Winkler method as described by Astor (2011). Detection limit

is about 5 μmoles per liter and precision is about 1 μmoles per liter. Data Quality Method: Comparison to known standards and historical data.

Nutrients
(CAR 108, 108B, 108C, 112, 112B, 112C, 118, 118B, 118C, 122, 122B, 122D, 128, 128B, 132, 132B, 132D, 139, 145, 153, 157, 163, 169, 175, 180, 186, 191, 201, 207, 212, 216, 224)

Nitrate
Nitrate concentrations were analyzed at the University of South Florida by Kent Fanning's on frozen samples using the method of Fanning et al (2011). Detection limit was 0.04 micromoles per liter. Samples for cruises 216 and 224 were performed by Kristen Buck's lab using the same method and the detection limit was 0.06 micromoles per liter. Data Quality Method: Comparison to known standards and historical data.

Nitrite
Nitrite concentrations were analyzed at the University of South Florida by Kent Fanning's lab on frozen samples using the method of Fanning et al (2011). Detection limit was 0.01 micromoles per liter. Samples for cruises 216 and 224 were performed by Kristen Buck's lab using the same method and the detection limit was 0.01 micromoles per liter. Data Quality Method: Comparison to known standards and historical data.

Ammonium
Ammonium concentrations were analyzed at the University of South Florida by Kent Fanning's lab on frozen samples using the method of Fanning et al (2011). Detection limit was 0.1 micromoles per liter. Samples for cruises 216 and 224 were performed by Kristen Buck's lab using the same method and the detection limit was 0.05 micromoles per liter. Data Quality Method: Comparison to known standards and historical data.

Phosphate
Phosphate concentrations were analysed at the University of South Florida by Kent Fanning's lab on frozen samples using the method of Fanning et al (2011). Detection limit was 0.02 micromoles per liter. Samples for cruises 216 and 224 were performed by Kristen Buck's lab using the same method and the detection limit was 0.02 micromoles per liter. Data Quality Method: Comparison to known standards and historical data.

H2S:
(CAR 1, 19, 25, 29, 32, 36, 42, 48, 54, 60, 66, 74,78, 89, 96, 100, 108, 108B, 108C, 118, 118B, 118C, 122, 122B, 122D, 128, 132, 132B, 132D, 112, 118, 122, 128, 132, 139, 145, 153, 157, 163, 169, 180, 191, 201, 207, 212, 216, 224)
Seawater samples for hydrogen 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 were 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 was dissolved and was analyzed spectrophotometrically by modification of the method of Cline (1969) as modified by Hayes et al (2006) and described by Li and Astor (2011). For CAR 180, samples were measured both at Stony Brook and Edimar. Following CAR191 sulfide was measured in Venezuela. Sulfide samples from both our geochemistry cruise and the CARIACO time series cruise are reported for CAR 180.

Detection limit for sulfide was about 0.6 micromole per liter. In the tables, precisions for samples with concentrations greater than 2 micromoles per liter which were better than 10% are uncolored, precisions between 10 and 20% are lightest blue and precisions worse than 20% are darker blue. For CAR 1 to 175, sulfide analyses were performed in Stony Brook University.

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.

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 cruises CAR 191 and following sulfide samples were collected during the biogeochemistry cruise and were analyzed at EDIMAR to avoid problems in shipping.

Data Quality Method: Comparison to laboratory prepared standards and historical data.

Methane (CH4):
(CAR 1, 5, 13, 25, 29, 32, 36, 42, 48, 54, 60, 66, 74, 78, 89, 96, 100, 108, 108B, 108C, 118, 118B, 118C, 122, 122B, 122D, 128, 128B, 132, 132B, 132D, 139, 145, 157, 163, 169, 175, 180, 186, 191, 201, 207, 212, 216, 224)
Methane samples were collected into 50 ml crimp seal vials with overflow of several volumes. Single samples were run through CAR 78, following which samples were taken in duplicate. Samples were poisoned by addition of 0.25ml of 10N NaOH solution to each vial and were sealed with a Teflon lined butyl rubber seal and an aluminum crimp. Care was taken to minimize trapping of an air bubble. CH4 was 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 one or more standard gas mixtures of methane in nitrogen. For CAR 1 - 145 one standard was used, for 157 to 186 two standards were used, and for CAR 191 through CAR 224 three standards were used. The detection limit was approximately 0.01 to 0.02 micromoles methane per liter. Precision was typically better than 5% for concentrations above 0.05 micromoles per liter. In the tables, precisions for samples with concentrations greater than 0.05 micromoles per liter, which were better than 10% are uncolored, precisions between 10 and 20% are lightest blue and precisions worse than 20% are darker blue. Data Quality Method: Comparison to known standards and historical data.

Sulfite and thiosulfate:
(CAR 108, 108B, 108C, 118, 118B, 118C, 122, 122B, 122D, 128, 128B, 132, 132B, 132D, 139, 145, 153, 157, 169, 175, 180, 186, 191)
Descriptions of the methods for these parameters also can be found in Hayes et al. (2006) and Percy et al. (2008). 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 0.2 moles/ liter sodium acetate buffer. 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.

The analytical detection limit (6x the standard deviation of five laboratory blanks) was 0.3 micromoles per liter for sulfite and 0.6 micromoles per liter for thiosulfate (Percy et al 2008). Field blanks were assumed to be lower than the lowest measured sample in a given cast. Upper estimates of the true blanks which are the lowest thiosulfate and sulfite value which were measured during a particular cruise were 0.8 micromoles per liter and 0.6 micromoles per liter, respectively in Percy et al (2008) and 0.6-0.8 and 1.5 to 2.2 micromoles per liter for Hayes et al. (2006). The precision of analysis (relative standard deviation of 5 replicates of a 10 micromoles per liter standard) for thiosulfate and sulfite was ± 2.2% and ± 1.6% respectively, although precision of replicates at lower concentrations typical of the Cariaco Basin are worse. The database includes standard deviations for triplicate or quadruplicate samples.

Data Quality Method: Comparison to laboratory prepared standards and historical data.

Particulate Elemental Sulfur:
(CAR 118, 122, 122B, 122D, 128, 132, 132B, 132D, 139, 145, 153, 157, 163, 169, 175, 180, 186, 191, 201, 207, 212, 216, 224)

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 2.2 min. Elemental sulfur was detected at 226 nm except for CAR 216 and 224 when detection was at 264 nm. (Experiments showed that no difference in concentration was obtained in using the two wavelengths).

The detection limit of about 0.1 micromoles per liter in a 200 ml seawater sample. Standard solutions, made by dissolving sulfur powder in methanol and serially diluting, are linear in the range of 1–100 µmol L^{-1}. The variability of replicates (triplicates) is given as standard error in the database.

Data Quality Method: Comparison to laboratory prepared standards and historical data.

Total Zero-valent Sulfur:
(CAR 128, 132, 139, 145, 153, 157, 175, 180, 186, 191, 201, 207, 212, 216, 224)
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 of sulfur standards to >90%. The pooled extraction was diluted 1:3 with methanol to optimize chromatography. We used a ODS hypersil C_{18} reverse phase, 250 mm × 4.6 mm, 5 µm column (Supelco Co.) at room temperature. 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. For TZVS for CAR 214 and 224, the column used was a Zorbax ODS C18 4.6 x 250 mm, 0.5 micrometer reverse phase HPLC column (samples run in November 2016) or a Zorbax Eclipse C18 4.6 x 150 mm, 0.5 um reverse phase HPLC column (samples run in April 2017). Samples run in November 2016 used a flow rate of 0.5 mL/min and peaks had a retention time of ~14 minutes. Samples run in April 2017 were run at a flow rate of 0.8 mL/min, also yielding a retention time ~ 14 minutes. For both CAR-216 and CAR-224, samples, standards were not diluted with methanol, only chloroform. Standard range was 0-30 micromole/L.

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.

Samples for CAR 224 were shaken for 60 minutes while all other TZVS samples were shaken for one minute.

The detection limit for TZVS was about 0.14 micromoles per liter. Variability of replicates is given as standard error in the database. Data Quality Method: Comparison to laboratory prepared standards and historical data.

Acetate
(CAR 1, 5, 9, 13, 19, 25, 29, 32, 36, 42)

Low molecular weight fatty acids: Volatile fatty acids are measured using the technique developed by Yang (1991), Yang et al. (1993) and Wu and Scranton (1994). Detection limits are about 1 micromoles per liter for acetate. However, in some cases, deep water values are lower than 1 micromolar for acetate, in which case we would take an upper limit of the blank from the lowest value measured. Data are not blank corrected except for CAR 42 (see below). Samples are poisoned with 1 ml 10N KOH per liter. Data Quality Method: Comparison to laboratory prepared standards and historical data.

Propionate
(CAR 5, 9, 13, 19)
Low molecular weight fatty acids: Volatile fatty acids are measured using the technique developed by Yang (1991), Yang et al. (1993) and Wu and Scranton (1994). Samples are poisoned with 1 ml 10N KOH per liter. Detection limits were not well determined for propionate but are about 200-300 nanomole per liter. Data are not blank corrected. Data Quality Method: Comparison to laboratory prepared standards and historical data.

Acetate uptake rate constant
(CAR 1, 5, 9, 13, 19, 25, 29, 32, 36, 42, 74. In addition either incorporation or respiration rate constants were made on CAR54, 60, 66)
Acetate uptake rate constants are determined using radiolabeled tracers as described by Wu and Scranton (1994) and Ho et al. (2002). Incubations are done anoxically in the dark in screw-top septum vials. Uptake includes both conversion of isotope to CO2 (respiration) and to biomass, which can be filtered onto a 0.2 m Nuclepore filter (incorporation). Uptake is calculated as measured concentration times rate constant. Data Quality Method: No authentic standards available. Data were corrected with killed controls and replicates lying outside 2 standard deviations are considered suspect.

Dissolved Iron
(CAR 108, 108B, 108C, 112, 112B, 112C, 118, 132, 132B, 132D, 139, 145)
Dissolved iron was determined as described by Percy et al. (2008). Analytical detection limit was about 2nM, and the field detection limit was about 50-100 nM. Because of the strong likelihood of contamination on the B/O Hermano Ginés, we only consider values from below the appearance of sulfide as detectable although all data are included in the database. Analytical Precision was 5.4% and field precision (based on duplicate samples) was 32%. Data Quality Method: Comparison to laboratory prepared standards and historical data.

Dissolved Manganese
(CAR 108, 108B, 108C, 112, 112B, 112C, 118, 132, 132B, 132D, 139, 145)
Dissolved iron was determined as described by Percy et al. (2008). Analytical detection limit 2nM, field detection limit at least 10 nM. Because of the strong likelihood of contamination on the B/O Hermano Ginés, we only consider values from below the appearance of sulfide as detectable. Data should be used with caution. Analytical Precision was about 4.7%; field precision 8%. Data Quality Method: Comparison to laboratory prepared standards and historical data.

Microbial census:
Censuses of total prokaryotes and flagellated protists were all performed on subsamples from 220 ml seawater samples that were preserved in the field with 2%

(final conc.) borate-buffered formaldehyde and stored in the dark at 5°C. All analyses were performed using variations of epifluorescence microscopy. Sample volumes passed through membranes were adjusted according to technique, depth and abundance of target organisms to obtain sufficient cell densities to optimize counting statistics. For most samples, ten random fields or grids were counted to achieve a total census exceeding 300 cells (Kirchman et al., 1982). In samples with lower cell densities per field, more fields were counted to exceed a 200 total cell threshold. With every batch of samples processed, a blank filter was prepared to correct for reagent and filtration apparatus contamination (subtracted from sample values). If blank values exceeded 5 cells per field, new reagents were prepared. Detection limits are controlled by cell concentration and available sample volume to filter and cannot be specified definitively, but were never approached with our samples. Ability to detect formalin-preserved microbes is known to decay with storage time, even at 5°C. To account for this, we analyzed subsamples from all depths from a single cruise after 1, 7, 16, 31, 49, 64, 95, 297, and 960 days of storage. This yielded the following exponential decay curve; Ns = Nc e-0.000605(ts – tc) for prokaryotes, where Ns = cell abundance at time of sample preparation (ts), Nc = cell abundance at time of sample collection in the field (tc), and -0.000605 d-1 is the empirical decay constant (slope of ln N vs time). All total prokaryote and flagellated protist abundance data were corrected for storage time elapsing between sample collection and preparation of stained filters using this decay constant. Accuracy and precision are difficult to specify as authentic standards and independent methods for comparison were not available. Precision is affected by natural biological variability, aggregation, and membrane irregularities. We address precision for each assay by computing the relative standard error for the entire time-series (SE/mean) below.

Total Prokaryote Cell Density:
(CAR 1, 5, 9, 13, 19, 25, 29, 32, 36, 42, 48, 54, 60, 66, 74,78, 89, 96, 100, 108, 112, 118, 108B, 108C, 112, 112B, 112C, 118, 118B 118C, 122, 122B, 122D, 128, 128B, 132, 132B, 132D, 139, 145, 153, 157, 163, 169, 175, 180, 186, 191, 201, 207, 212, 216, 224)

Samples (200-225 ml) were preserved shipboard with 2% borate-buffered formaldehyde in 250 ml polyethylene bottles and refrigerated until analyzed. Subsamples of 10-20 ml were either stained with acridine orange or DAPI and captured on 0.2 μm polycarbonate membranes (Hobbie et al. 1977; Porter & Feig 1980). Ten or more microscopic fields were counted at 100x magnification on each filter to achieve a total census of cells exceeding 300 (Taylor et al., 2001). Mean relative standard error for this assay was ±9% over the entire time-series. Data Quality Method: No authentic standards available. Data were corrected with reagent blank membranes. Samples exhibiting anomalous results relative to temporal and vertical trends were resampled, i.e., complete sample preparation was repeated to support or refute validity of results. Ability to detect formalin-preserved microbes is known to decay with storage time, even at 5°C. To account for this, we analyzed subsamples from all depths from a single cruise after 1, 7, 16, 31, 49, 64, 95, 297, and 960 days of storage. This yielded the following exponential decay curve; Ns = Nc e-0.000605(ts – tc) for prokaryotes, where Ns = cell abundance at time of sample preparation (ts), Nc = cell abundance at time of sample collection in the field (tc), and -0.000605 d-1 is the empirical decay constant (slope of ln N vs time). All total prokaryote and flagellated protist abundance data were corrected for storage time elapsing between sample collection and preparation of stained filters using this decay constant.

Total Prokaryote Carbon Biomass Estimates:
(CAR 1, 5, 9, 13, 19, 25, 29, 32, 36, 42, 48, 54, 60, 66, 74,78, 89, 96, 100, 108, 112, 118, 108B, 108C, 112, 112B, 112C, 118, 118B 118C, 122, 122B, 122D, 128, 128B, 132, 132B, 132D, 139, 145, 153, 157, 163, 169, 175, 180, 186, 191, 201, 207, 212, 216, 224)

Prokaryotic cell sizes in a subset of all samples (n = 215 samples) were estimated from 200 randomly selected fluorescent cells in each sample by visually sorting into seven morphology/size classes based on their linear dimensions, approximated with an ocular micrometer at 1000x magnification. Mean biovolumes of each of the eight size classes were estimated using the following formula: V = (p/4)*W^2*(L-W/3), where V is cell volume (µm^3), L (µm) is cell major axis, and W (µm) is cell minor axis (Bratbak 1985). The sum of all cell volumes at each depth is divided by the total cell counts to obtain mean biovolume (µm^3 cell^-1). Binning of observed cells into size classes was facilitated by entering data on an 8-place mechanical counter. Cellular carbon biomass (C) was estimated from biovolume (V) using an allometric carbon to volume extrapolation function, pg C = 0.12 V^0.72 (Norland, 1993). Allometric extrapolations appear to be more accurate than linear conversions when cell volumes are larger than 0.2 µm^3 (Posch et al., 2001). We found that the Norland (1993) conversion yields values agreeing well with literature values of direct elemental analysis of prokaryoplankton (Gunderson et al., 2002). Among all sized samples, mean cell biovolumes were found to consistently vary among samples collected from 0-100, 101-250, 251-450, and >450 meters, but not significantly vary among seasons (ANOVA, p > 0.1). Therefore, in all remaining samples, mean cellular carbon biomass conversion factors of 32.6, 34.4, 35.6, and 48.1 x 10^-15 g C cell^-1 were applied to measured cell concentrations collected from 0-100, 101-250, 251-450, and >450 meters, respectively.

Flagellated Protists:
(CAR 1, 5, 9, 13, 19, 29, 32, 36, 42, 48, 78, 108, 112, 112B, 122, 122B, 122D, 128, 128B, 139, 145, 153, 157, 169, 175, 180, 186, 191, 201, 207, 212, 216, 224)

Samples (200-225 ml) were preserved shipboard with 2% borate-buffered formaldehyde in 250 ml polyethylene bottles and refrigerated until analyzed. Subsamples (20-50 ml) were stained with acridine orange captured on 0.8 µm polycarbonate membranes (Hobbie et al. 1977). Twenty or more microscopic fields at 63x magnification were counted on each filter to achieve a total census of cells exceeding 200. Mean relative standard error for this assay was ±16% over the entire time-series. Data Quality Method: No authentic standards available. Data were corrected with reagent blank membranes. Samples exhibiting anomalous results relative to temporal and vertical trends were resampled, i.e., complete sample preparation was repeated to support or refute validity of results. Ability to detect formalin-preserved microbes is known to decay with storage time, even at 5°C. To account for this, we analyzed subsamples from all depths from a single cruise after 1, 7, 16, 31, 49, 64, 95, 297, and 960 days of storage. This yielded the following exponential decay curve; Ns = Nc e-0.000605(ts – tc) for prokaryotes, where Ns = cell abundance at time of sample preparation (ts), Nc = cell abundance at time of sample collection in the field (tc), and -0.000605 d-1 is the empirical decay constant (slope of ln N vs time). All total prokaryote and flagellated protist abundance data were corrected for storage time elapsing between sample collection and preparation of stained filters using this decay constant.

Ciliated Protists:
(CAR 5, 9, 13)
Samples (200-225 ml) were preserved shipboard with 2% borate-buffered formaldehyde in 250 ml polyethylene bottles and refrigerated until analyzed. Subsamples (150 ml) were stained with acridine orange captured on 2.0 μm polycarbonate membranes. Based on size and gross morphological features, ciliates were enumerated separately to the class or sub-order taxonomic levels. (Taylor et al. 2006). Twenty or more microscopic fields at 40x magnification were counted on each filter to achieve a total census of cells exceeding 150. Mean relative standard error for this assay was ±13% over the entire time-series. Data Quality Method: No authentic standards available. Data were corrected with reagent blank membranes. Samples exhibiting anomalous results relative to temporal and vertical trends were resampled, i.e., complete sample preparation was repeated to support or refute validity of results.

Cyanobacteria:
(CAR 1,5,9,13)
Samples (200-225 ml) were preserved shipboard with 2% borate-buffered formaldehyde in 250 ml polyethylene bottles and refrigerated until analyzed. Subsamples (10-20 ml) were captured on 0.2 μm polycarbonate membranes and not stained. Cyanobacterial cells were observed to autofluoresce using Zeiss' standard Rhodamine excitation/emission filter set. (MacIsaac & Stockner 1993). Ten or more microscopic fields were counted at 100x magnification on each filter to achieve a total census of cells exceeding 300. Mean relative standard error for this assay was ±8% over the entire time-series. Data Quality Method: No authentic standards available. Data were corrected with reagent blank membranes. Samples exhibiting anomalous results relative to temporal and vertical trends were resampled, i.e., complete sample preparation was repeated to support or refute validity of results.

Methanogenic Archaea:
(CAR 1, 5, 9)
10-20 ml samples were captured on 0.2 μm polycarbonate membranes and not stained. Methanogenic cells were observed to autofluoresce using Zeiss' custom F420 excitation/emission filter set. F420 is a coenzyme that participates in methanogenesis (Doddema & Vogels 1978). Mean relative standard error for this assay was ±7% over the entire time-series.

Viral-Like Particles (VLP):
(CAR 19, 25, 29, 32, 36, 42, 207, 212, 224)
Samples (200-225 ml) were preserved shipboard with 2% borate-buffered formaldehyde in 250 ml polyethylene bottles and refrigerated until analyzed. Viral-like particles in 0.3 to 2 ml subsamples were captured on 0.02 μm Anodisk 25 mm membranes (Whatman) were stained for 15 min under darkness with SYBR Green I fluorochrome (Molecular Probes) (Noble & Fuhrman, 1998; Taylor et al. 2003). Ten or more microscopic grids were counted at 100x magnification on each filter to achieve a total VLP census exceeding 300. Mean relative standard error for this assay was ±16% over the entire time-series. Data Quality Method: No authentic standards available. Data were corrected with reagent blank membranes. Samples exhibiting anomalous results relative to temporal and vertical trends were resampled, i.e., complete sample preparation was repeated to support or refute validity of results.

Heterotrophic Bacterial Production:
(CAR 1, 5, 9, 13, 19, 25, 29, 32, 36, 42, 48, 54, 60, 66, 74,78, 89, 96, 100, 108, 112, 118, 108B, 108C, 112, 112B, 112C, 118, 118B 118C, 122, 122B, 122D,

128, 128B, 132, 132B, 132D, 139, 145, 153, 157, 163, 169, 175, 180, 186, 191, 201, 207, 212, 216, 224)

Bacterial heterotrophic production, often called "Bacterial Net Production (BNP)" was measured using ^{3}H-leucine incorporation into cellular protein as described by Kirchman (1993). Triplicate samples were incubated for 10-12 h in gas-tight screw-top septa vials to minimize alteration of the redox potential. Time course experiments 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. Data Quality Method: Detection limits of this assay as employed were 0.01 μg C L^{-1} d^{-1} and assay accuracy cannot be determined as there is no independent standard for calibration. As an indicator of precision, mean relative standard error for this assay was ±19% over the entire time-series.

Dark carbon fixation
(CAR 13, 19, 25, 29, 32, 36, 42, 48, 54, 60, 66, 74,78, 89, 96, 100, 108, 112, 118, 108B, 108C, 112, 112B, 112C, 118, 118B 118C, 122, 122B, 122D, 128, 128B, 132, 132B, 132D, 139, 145, 153, 157, 163, 169, 175, 180, 186, 191, 201, 207, 212, 216, 224)

Chemoautotrophic assimilation of inorganic carbon was measured by ^{14}C-bicarbonate incorporation into particles (Taylor et al. 2001). For cruises CAR 13 through CAR 29 samples were incubated in Pierce septa vials with Teflon lined butyl rubber septa. Due to concern about oxygen transfer across the septa, for CAR 25 and CAR-29 we compared rates using Pierce vials and ground glass stoppered vials across the redoxcline and found little difference, but following CAR 29 all samples were incubated in ground glass stoppered vials. After dispensing samples into 40-ml bottles, 200 ml of chilled N_2-purged ^{14}C-bicarbonate in an alkaline brine (pH 9.5; S = 60 on the practical salinity scale) was injected into the bottom before sealing (Tuttle and Jannasch 1973a. Samples were incubated underwater at approximately ambient temperature in the dark for 14–20 h. Time-course experiments showed rates were linear up to 30 h. Particles were collected on 0.22 mm cellulosic membranes (Osmonics), which were then rinsed twice with 5 ml of filtered seawater. Filters were purged of unassimilated inorganic ^{14}C in a saturated HCl atmosphere for more than 1 hour, then were dried and suspended in Hionic-Fluor scintillation cocktail and radioassayed. Data were corrected for isotopic fractionation (multiplied by 1.06) and for nonbiological sorption by use of samples processed immediately after introduction of the radiotracer. Rates of dark ^{14}C-assimilation were normalized to micromoles carbon per day by use of values of dissolved inorganic carbon (DIC) derived from pH, temperature, and alkalinity measurements from the time series cruises. Data Quality Method: Detection limits of this assay as employed were 0.01 μg C L^{-1} d^{-1} and assay accuracy cannot be determined as there is no independent standard for calibration. As an indicator of precision, mean relative standard error for this assay was ±27% over the entire time-series.

Specific comments on individual cruises

CAR 1, CAR-5, CAR-9 and CAR-13: Locations for individual casts not recorded. Station location is that indicated on ship's log. Following this we recorded the location at the time each cast reached depth.

CAR 25: Corrected depth 15: For this sample, the intended depth was 15 m. However, the CTD record shows no signs of a sample having been taken at 15 m. We have flagged this depth in yellow.

CAR 29 Corrected depths not available. Intended depths are tabulated instead. These depths are marked in yellow.

CAR 42: For this cruise, a blank of 2 micromoles per liter has been subtracted from acetate concentrations measured by GC

CAR 60: Corrected depths not available for cast 1 and for 1300 m on cast 3. Intended depths are tabulated instead. These depths are marked in yellow.

CAR 132A and CAR 132B: Methane values for CAR-132A and CAR 132B are noticeably lower than cruises before or after. There may have been storage or septum leakage problems.

CAR 157: Although silicone sealant appeared to cure correctly, values for this date are surprisingly low. Contact Scranton for more discussion

CAR 163: Deep water H2S sample (500, 900, 1300 m) were not diluted to bring the absorbance into the linear portion of the calibration curve. Values may be slightly underestimated.

CAR 169: Methane values may be low. Silicone sealant poorly cured. Higher variation in replicates for deep water H2S samples than usual is probably related to incomplete mixing of samples after adding diamine

CAR 175: Silicone sealant used to seal holes in septa on methane samples poorly cured. Total zero valent sulfur seem high and surprisingly constant especially above interface. However data have been included here as we have less experience with this parameter. Contact Scranton for further discussion.

CAR 180: Sulfide data were calculated based on Na2S.11H2O as calibrated by Lan Tong with Winkler back titration on 11 July 2011

CAR 186: H2S samples for biogeochemistry cruise were lost due to leaking during transportation . Silicone sealant used to seal holes in methane septa poorly cured.

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