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Chemoautotrophy in the redox transition zone of the Cariaco Basin: A significant midwater source of organic carbon production

Gordon T. Taylor,[1] *Maria Iabichella, Tung-Yuan Ho, and Mary I. Scranton*
Marine Sciences Research Center, State University of New York, Stony Brook, New York 11794-5000

Robert C. Thunell
Department of Geological Sciences, University of South Carolina, Columbia, South Carolina 29208

Frank Muller-Karger
Department of Marine Sciences, University of South Florida, St. Petersburg, Florida 33701

Ramon Varela
Estacion de Investigaciones Marinas de Margarita, Fundacion la Salle de Ciencias Naturales, Apartado 144, Punta de Piedras, Edo. Nueva Esparta Venezuela

Abstract

During the CARIACO time series program, microbial standing stocks, bacterial production, and acetate turnover were consistently elevated in the redox transition zone (RTZ) of the Cariaco Basin, the depth interval (~240–450 m) of steepest gradient in oxidation-reduction potential. Anomalously high fluxes of particulate carbon were captured in sediment traps below this zone (455 m) in 16 of 71 observations. Here we present new evidence that bacterial chemoautotrophy, fueled by reduced sulfur species, supports an active secondary microbial food web in the RTZ and is potentially a large midwater source of labile, chemically unique, sedimenting biogenic debris to the basin's interior. Dissolved inorganic carbon assimilation (27–159 mmol C m^{-2} d^{-1}) in this zone was equivalent to 10%–333% of contemporaneous primary production, depending on the season. However, vertical diffusion rates to the RTZ of electron donors and electron acceptors were inadequate to support this production. Therefore, significant lateral intrusions of oxic waters, mixing processes, or intensive cycling of C, S, N, Mn, and Fe across the RTZ are necessary to balance electron equivalents. Chemoautotrophic production appears to be decoupled temporally from short-term surface processes, such as seasonal upwelling and blooms, and potentially is more responsive to long-term changes in surface productivity and deep-water ventilation on interannual to decadal timescales. Findings suggest that midwater production of organic carbon may contribute a unique signature to the basin's sediment record, thereby altering its paleoclimatological interpretation.

The permanently anoxic Cariaco Basin on the northern continental margin of Venezuela (Fig. 1) has been treated by oceanographers, paleoceanographers, and paleoclimatologists as a natural sediment trap, recording climatic changes in the tropical Atlantic region over timescales varying from seasons to the last 12,000 yr (Hughen et al. 1996). The basin's varved sediments consist of alternating layers of light biogenic debris (formed by upwelling-driven plankton blooms during the dry, windy season) and dark continental materials deposited during the wet season (Overpeck 1989; Peterson et al. 1991; Hughen et al. 1996). Relative thicknesses of biogenic layers are believed to reflect the intensity and duration of upwelling, its accompanying plankton blooms, and vertical export to the seabed. Stable isotope and biomarker signatures in sediments have been interpreted as further indicators of upwelling intensity, planktonic community structure, and trophic status (Werne et al. 2000). However, inferences from the sedimentary record are based on the assumptions that delivery of biogenic debris to the seabed is exclusively driven by surface processes and that source and decay terms are well known.

In open waters, deposition of biogenic debris is adequately understood as a function of depth, surface productivity, epipelagic community structure, and aerobic remineralization of material in transit (Pace et al. 1987; Michaels and Silver 1988; Taylor 1989). However, lateral advection of surface or intermediate waters in open systems and bioturbation by the

[1] Corresponding author (gtaylor@notes.cc.sunysb.edu).

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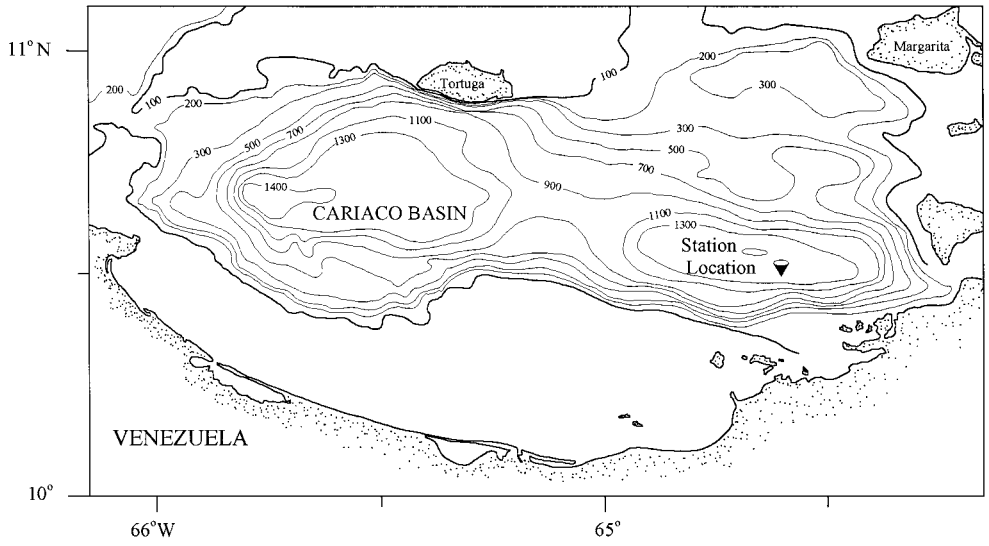

Fig. 1. Map of the Cariaco Basin in the Caribbean Sea and location of the time series Sta. CARIACO (10.50°N, 64.66°W). The Cariaco system consists of two sub-basins (east and west) connected by a saddle at a depth of ~900 m. Bathymetric contours are presented in meters.

benthos may corrupt the sedimentary record's representation of overlying epipelagic processes. In contrast, the enclosed and stratified Cariaco Basin would appear to be an ideal site to examine the relationship between export flux and sediment deposition, because of the basin's limited lateral exchange and its anoxia. This large, tectonically formed basin (approximate volume below 180 m, 5.2×10^{12} m^3) is 1,400 m deep, is surrounded by a sill (90–150 m in depth), and has remained anoxic below depths of 250–350 m for centuries (Richards 1975). In effect, its geomorphology confines lateral advection to the surface layer, and biogenic, locally generated debris primarily accumulates in the basin's sediments (Thunell et al. 2000). Thus, these sediments potentially represent excellent integrators of surface processes for the region. Furthermore, integrity of depositional layers is exceptionally good because of the system's physical quiescence and the absence of bioturbation caused by metazoan infauna.

Stratified water columns prone to anoxia have long been known to support multiple layers of biological production (Sorokin 1972; Indrebø et al. 1979; Jørgensen et al. 1979, 1991; Sorokin et al. 1995). Chemical gradients of electron donors and acceptors are established at depth by anaerobic mineralization of biogenic debris exported from the photic zone. Large and productive microbial communities at the oxic-anoxic interface capitalize on the residual chemical energy (H_2S, NH_4^+, CH_4, H_2, low-molecular-weight organics, etc.) emanating from anoxic waters. Enrichments in bacterial abundances, microbial adenosine triphosphate (ATP), protozoa, elemental cycling, and chemoautotrophic production have been reported for the oxic-anoxic interface of the Cariaco Basin and the Black Sea, the two largest examples of such systems (Sorokin 1972; Karl et al. 1977; Karl 1978; Hastings and Emerson 1988; Bird and Karl 1991). The extent to which activity in these layers is coupled to surface processes, the magnitude of energy recovery, and the contribution of this zone to a secondary, midwater flux of biogenic debris are unknown. These processes could have a significant impact on the sediment record and its interpretation.

The present investigation reports on midwater carbon-flux anomalies and the microbial processes that may be their source in the Cariaco Basin. In the past, this system has received sporadic study, but it recently (Nov 1995 to the present) has been the site of an intensive time-series program. The cooperative U.S.-Venezuelan Carbon Retention in a Colored Ocean (CARIACO) program consists of monthly Joint Global Ocean Flux Study–style productivity cruises and seasonal process cruises, continuous meteorological and remote sensing monitoring, and sediment flux measurements from an array of moored sediment traps. Results from this time series confirm that midwater enrichments in microbiological standing stocks and activities are a persistent feature in this dynamic system. Moreover, our observations suggest that bacterial chemoautotrophic production within the redox transition zone (RTZ) contributes significantly to overall biological productivity, governs elemental cycling across the oxic-anoxic interface, and may influence the chemical quality of the sedimentary flux.

Materials and methods

Site description and sampling—The CARIACO time-series station is located in the eastern subbasin of the Cariaco system (Fig. 1) in nearly 1,400 m of water (10.50°N, 64.66°W). All results presented are from this single site. Monthly sampling was conducted aboard the B/O *Hermano Gines,* operated by Estacion de Investigaciones Marinas, Fundacion la Salle de Ciencias Naturales, located on Margarita Island, Venezuela. During process cruises, conducted 2–3 times per year, water samples were collected at 18 depths with a SeaBird rosette accommodating 12 TFE (tetrafluoroethylene, Teflon)-lined, 8-liter Niskin bottles. For

hydrographic profiling, the rosette included a SeaBird conductivity, temperature, depth (CTD) probe, a Yellow Springs Instruments, Inc. oxygen probe, a Chelsea Instruments fluorometer for chlorophyll *a* estimates, and a Sea Tec c-beam transmissometer (660 nm). To resolve relatively narrow features, vertical sampling intervals were 10–20 m across the oxic-anoxic interface and greater than that in shallow and deep waters. Peaks in beam attenuation from the transmissometer were found to be reliable proxies for bacterial maxima near the interface, and sampling depths were adjusted accordingly, to resolve these features. Time, manpower, and the ship's motion, however, constrained sampling resolution, so finer-scale features were sometimes missed. Samples were withdrawn from Niskin bottles under N_2 atmosphere, to prevent oxygenation of samples. All samples used for biological rate measurements were transferred from Niskin bottles to HCl-washed 1-liter TFE-stoppered glass bottles and sealed without head space after overflowing ~1–2 volumes. Samples for biological incubations were then dispensed under N_2 pressure from these 1-liter bottles into acid-washed, 40-ml septa vials (laminated TFE-butyl rubber septa; Pierce) or into 40-ml glass-stoppered bottles and sealed without head space after overflowing.

Microbial abundances and heterotrophic production—At each depth, whole-water samples (200 ml) were preserved with 2% (final concentration) borate-buffered formaldehyde and stored at 5°C. In the laboratory, standard DAPI (4′6-diamidino-2-phenylindole)-stained slides were prepared on dark 0.2 or 0.8 μm Poretics polycarbonate membranes for enumeration, by epifluorescence microscopy, of bacteria or flagellated protozoa, respectively (Porter and Feig 1980). Viral-like particles (VLPs) were enumerated by epifluorescence microscopy according to the methods of Noble and Fuhrman (1998).

Bacterial net production (BNP) was estimated from incorporation of ^{3}H-leucine into protein, according to the methods of Kirchman (1993). At each depth, triplicate samples in 40-ml septa vials were immediately spiked with 50 μl of N_2-purged ^{3}H-leucine [10 nM final concentration; L-(4,5-^{3}H[N])-leu; 52 Ci mmol^{-1}] using a gas-tight syringe. Samples were incubated in on-deck water baths for 8–12 h and maintained at ambient temperature in darkness. Samples from the interface and below were incubated in sulfidic deep waters maintained at 17–19°C and shallow samples in surface water at 25–28°C. After incubation, samples were fixed with cold trichloroacetic acid (TCA; 5% final concentration) and refrigerated until processing immediately after the cruise according to the methods of Kirchman (1993). ^{3}H-leucine incorporated into protein over time was corrected with filter blanks (T_0) from samples fixed with 5% TCA immediately after ^{3}H-leucine was added. BNP was calculated using a conversion factor of 3.1 kg C mol^{-1} of leucine incorporated (Kirchman 1993).

Acetate turnover—Acetate uptake rates were estimated by calculating rate constants of ^{14}C-acetate incorporation and respiration and multiplying their sum by ambient acetate concentrations (Wright and Hobbie 1966; Hobbie and Crawford 1969). Acetate uptake rate constants were determined by immediately spiking samples in septa vials (as described above) with 100 μl of N_2-purged ^{14}C-acetate (<1 nM final concentration; 100 Ci mmol^{-1}, uniformly labeled). During time courses (0–12 h), individual vials were sacrificed, and duplicate subsamples (5 ml) were collected on 0.2 μm Nuclepore polycarbonate membranes, to measure incorporation. The remaining sample was alkalized with 0.5 ml 10N KOH, to kill biota and minimize $^{14}CO_2$ loss. Respired ^{14}C-acetate was measured later in the lab by acidification of the alkalized sample in a closed flask containing a suspended filter soaked in 2N KOH. Filters and $^{14}CO_2$ traps were radioassayed in Optiflour scintillation liquid cocktail. Rate constants were calculated as the sums of the slopes of incorporated and respired ^{14}C plotted against time. This approach corrects for passive sorption of ^{14}C-acetate to particles or membranes, background $^{14}CO_2$ in the spike, and any volatilization of ^{14}C-acetate during trapping of $^{14}CO_2$, which should be greatest at the initiation of experiments.

Immediately after hydrocasts (usually within 30 min), samples (250 ml) for ambient acetate concentration were passed through 0.2-μm Nuclepore membranes at moderate (<200 mm Hg) vacuum pressure. To stop microbial activity, 0.5 ml of 10N KOH was added to each filtered sample. Acetate and other low-molecular-weight fatty acids were preconcentrated by use of a static diffusion technique (Yang et al. 1993). Concentrations were corrected for recovery efficiency, which was determined by addition of a ^{14}C-acetate internal standard, and for reagent blanks. Preconcentrated samples were analyzed with gas chromatography by using an HP FFAP 530μm bore fused silica column and FID (flame ionization detection) (Hordijk et al. 1990).

Vertical carbon flux—A sediment trap mooring was located in the deepest portion of the eastern basin (~1,400 m) and consisted of four automated traps positioned at depths of ~275, 455, 930, and 1,255 m (Thunell et al. 1999). Traps have a 0.5-m^2 opening at the top and 13 collection cups at the bottom, each programmed to sequentially collect samples over 2-week intervals. Prior to deployment, collection cups were filled with buffered formalin (2%) in filtered seawater, to preserve accumulating organic matter. Upon retrieval, collection cups were sealed and refrigerated. Particulate organic carbon concentrations were measured in a Perkin-Elmer 2400 Elemental Analyzer according to the methods of Froelich (1980).

Dark carbon assimilation—Chemoautotrophic assimilation of inorganic carbon was measured by ^{14}C-bicarbonate incorporation into particles. After dispensing samples into 40-ml ground glass–stoppered bottles, 200 μl 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 1973*a*). Samples were incubated in parallel with BNP and ^{14}C-acetate turnover samples for 14–20 h. Time-course experiments showed rates were linear up to 30 h (not presented). Particles were collected on 0.22 μm cellulosic membranes (Osmonics), which were then rinsed twice with 5 ml of filtered seawater. Filters were purged of unassimilated ^{14}C in a saturated HCl atmosphere for >1 h, then dried and suspended in Hionic-Fluor

scintillation cocktail and radioassayed. Data were corrected for isotopic fractionation (×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 μM C d^{-1} by use of values of dissolved inorganic carbon (DIC) derived from pH, temperature, and alkalinity measurements (courtesy of Richard Bohrer, USF).

Primary production—Photosynthetic assimilation of inorganic carbon was measured by standard ^{14}C-bicarbonate protocols (UNESCO 1994). Samples were routinely collected from 1, 7, 15, 25, 35, 55, 75, and 100 m before dawn. Subsamples were dispensed under subdued light into 300-ml polycarbonate bottles—one dark and three transparent. Each bottle was spiked with 3.2–3.8 μCi of ^{14}C-bicarbonate and sealed. Bottles were deployed at ~0700 h local time on a buoyed array at their collection depth for a 4-h exposures to ambient light fields, equivalent to 33% of total daily irradiance at this latitude. After recovery, samples were filtered through Whatman GF/F filters, which were rinsed with 0.25 ml of 0.48N HCl, placed in scintillation vials, and radioassayed in Cytoscint scintillant. Data were corrected for isotopic fractionation (×1.06) and dark assimilation. Daily photosynthetic rates were estimated from measured hourly rates, photoperiod, and DIC concentrations (UNESCO 1994).

Dissolved inorganic chemical species—In addition to continuous dissolved O_2 concentration profiles obtained from the rosette's YSI electrode, O_2 in discrete samples was measured by standard Winkler titrations after samples were fixed in the field (Aminot 1983). Samples for H_2S were collected by syringe, avoiding atmospheric contact, and immediately transferred to vials containing zinc acetate or zinc chloride, to form ZnS precipitate. ZnS was derivatized and measured colorimetrically according to the methods of Cline (1969). NO_2^-, NO_3^-, and NH_4^+ concentrations were determined, by standard colorimetric methods, from frozen samples (Strickland and Parsons 1972). Mn and Fe were measured by graphite furnace atomic absorption spectrometry in filtered (0.2 μm) and unfiltered acidified samples to calculate particulate (presumably mostly oxidized) and dissolved (presumably mostly reduced) metal by difference (Balistrieri et al. 1992).

Results and discussion

Anomalies in vertical fluxes of biogenic debris—In open ocean and continental margin systems, carbon fluxes generally can be described by a power function of the form $f = b_0 Z^{-b1} \mathrm{NPP}^{b2}$, where f is the carbon flux (g C m^{-2} d^{-1}), b_0, b_1, and b_2 are empirically derived coefficients, Z is depth (in meters), and NPP is the net primary production (g C m^{-2} d^{-1}) (Pace et al. 1987) (curves in Fig. 2a). Vertical profiles of carbon fluxes at the CARIACO time-series station usually conform to these open-water models, and the hatched bars in Fig. 2a serve as an example. However, anomalies in sedimentation of biogenic debris are common, whereby carbon fluxes to the 455-m sediment trap exceed those to the 275-m trap by a significant margin, as exemplified by the solid bars in Fig. 2a. To illustrate the central tendencies and anomalies in depth-dependent decay, carbon fluxes to 455, 930, and 1,255 m were normalized by contemporaneous fluxes to 275 m for data collected from 8 Nov 95 to 24 Apr 99 (n = 71). Compare these data with curves derived from four published models, for open meso- to oligotrophic waters (Suess 1980; Betzer et al. 1984; Pace et al. 1987; Taylor and Karl 1991) (Fig. 2b). These models predict that fluxes to 455, 930, and 1,255 m should decrease to between 62% and 75%, 32% and 50%, and 24% and 43% of fluxes to 275 m, respectively.

The median values of our observations (vertical lines within box plots, Fig. 2b) agree well with predictions, demonstrating that, contrary to Demaison and Moore's (1980) assertion, decomposition in anoxic systems is not necessarily slower than that in oxic systems. Relatively high bottom temperatures in the Cariaco Basin (>17°C) may compensate to some extent for the absence of oxygen. Being temperature dependent, bacterial metabolism increases by factors of 2–3 for every 10°C increment (Q_{10}) until maximal rates are reached. Therefore, anaerobes in the Basin are expected to remineralize carbon at least twice as fast as anaerobes at comparable depths outside the basin, where temperatures are ~5°C. In fact, the mean (solid profile, Fig. 2b) and ~75% of all observations fall within the uncertainty of existing models' predictions (boxes, Fig. 2b). However, fluxes to 455 m exceeded fluxes to 275 m in 16 of the 71 observations, sometimes doubling between these depths (circles, Fig. 2b). These anomalies illustrate that vertical fluxes of biogenic debris leaving the RTZ may exceed export from surface waters (<275 m). A similar anomaly was suggested by a single set of observations in the Black Sea (Karl and Knauer 1991). The three most plausible mechanisms that could produce these observations (vertical migrators, lateral advection, and in situ production) are explored below.

Vertical migrators—Vertically migrating mesozooplankton and nekton feeding in surface waters, then defecating, molting, or dying below 275 m would enrich observed fluxes to 455-m traps. However, significant activity of metazoans, especially vigorous swimmers, is unexpected in this sulfidic system. For example, Vinogradov et al. (1985) found that O_2 concentrations of 18–22 μM in the Black Sea represented the lower boundary for vertical distributions of a variety of zooplankton, including copepods, ctenophores, and chaetognaths. O_2 concentrations usually fall below 20 μM near 200 m in the Cariaco Basin, which is well above the shallowest sediment trap. Nonetheless, diel vertical migrations of the Gadiformes fish, *Bregmaceros nectabanus*, across the Cariaco Basin's O_2-H_2S interface to depths of 800 m have been documented (Baird et al. 1973). During the CARIACO time series, the ship's fish finder repeatedly recorded an acoustic scattering layer that disappeared from surface waters at dawn, descended to depths >600 m by midday, and returned to surface waters after dusk (CARIACO program, unpubl. data). Presumably, the migrating scattering layer is caused by nekton or mesozooplankton. However, the identity of these populations has not been confirmed, and their abundance and behavior at depth remain unexamined. Recognizable feces or remnants from mesozooplankton or fish have

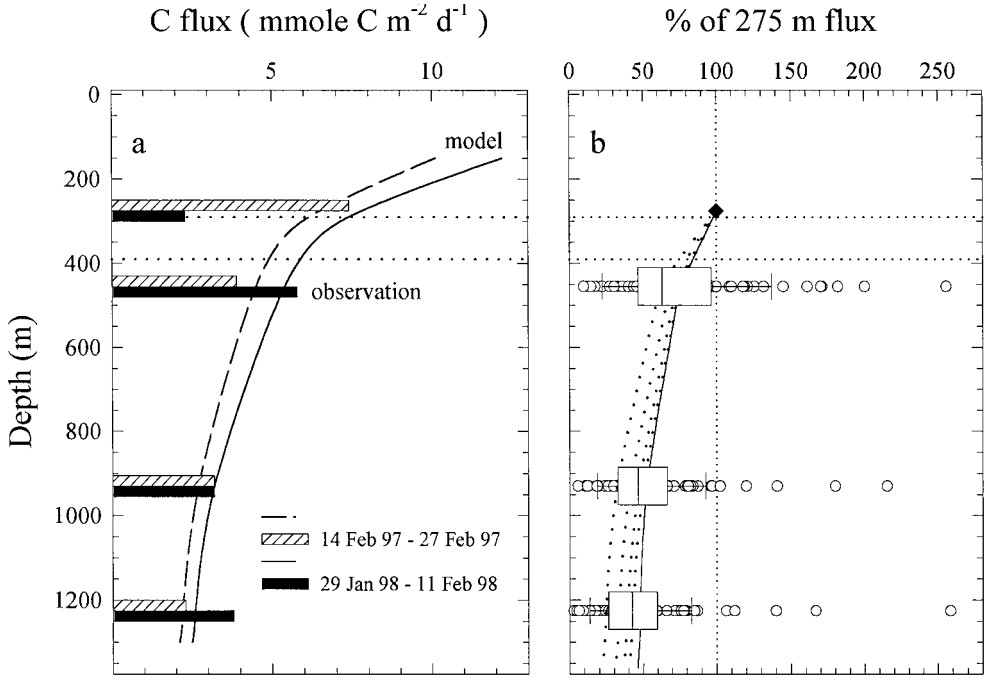

Fig. 2. (a) Examples of biogenic carbon fluxes collected over 2-week intervals in four moored, automated sediment traps at Sta. CARIACO from 14 to 27 Feb 97 and 29 Jan to 11 Feb 98. Curves represent carbon fluxes (*f*) predicted for the same time intervals, based on measured primary productivity (PP) and the equation $f = Z^{-0.734}\mathrm{PP}^{1.00}$, where *Z* is depth in meters (Pace et al. 1987). (b) Illustration of depth-dependent decomposition of biogenic carbon in the Cariaco Basin's interior. Fluxes of particulate carbon to 455, 930, and 1255 m are expressed as percentage of contemporaneous fluxes to 275 m (diamond). Solid profile represents the mean of 71 biweekly observations. Boxes delineate 75% of all observations and internal lines are the medians. Whiskers are the 10th and 90th percentiles, and circles represent individual observations. Dotted profiles are predictions based on empirical models of Suess (1980), Betzer et al. (1987), Pace et al. (1987), and Taylor and Karl (1991). Horizontal dotted lines define the redox transition zone in both panels.

never been observed in sediment trap materials below 275 m, so the impact of vertical migrators to flux anomalies is not immediately evident.

Lateral advection—A second possibility is that lateral advection may cause sedimentation anomalies. Lateral advection of water could bias sediment-trap observations by two independent mechanisms. If current speeds exceed a threshold value characteristic of the sediment trap's design, then traps would undersample as a result of internal turbulence and resuspension of material from the cone (Baker et al. 1988). If currents at 275 m sporadically exceed this undetermined threshold, then traps at this depth would collect at lower efficiencies than traps at 455 m and below, which presumably reside in quiescent water. This would result in an apparent flux anomaly. However, currents sufficient to generate turbulence have not been documented below sill depth, and hydrographic profiles are inconsistent with strong currents.

Lateral advection of biogenic debris from a more productive region to depths below 275 m would also produce flux anomalies. The extent of lateral intrusions of water masses below 275 m is constrained by a sill whose mean depth is 100 m (Richards 1975), and waters must sink at least 175 m prior to arriving at our station. Distributions of physical properties, salinity, temperature, and water density, do not provide evidence of intrusions, being nearly homogenous below 200 m for most of our monthly observations. For example, on the 7 Jul 98 (CAR-32) cruise, σ_θ increased monotonically from 26.390 to 26.446 between 200 and 455 m (Fig. 3a). However, water outside the basin is oxic at all depths, and if it were to spill over the sill and sink along isopycnals, then an oxygen anomaly would be predicted. Shallow O_2 anomalies (<200 m) are quite common (*see Fig. 3b*) and are evidence of lateral intrusions. Occasionally, such O_2 anomalies have been observed as deep as 340 m (Scranton et al. in press), but this is atypical, and their timing does not correspond with vertical flux anomalies (discussed below). Typically, dissolved O_2 distributions immediately above the interface (250–350 m) are smooth, as if they are controlled by diffusive supply from above and in situ consumption. Occasional intrusions of particle-rich waters may contribute to the basin sediment budget along the margin, but loadings of particles apt to sink in waters passing over our sediment traps are probably quite attenuated in comparison with near the sill, >50 km away. We conclude that the likelihood of lateral transport of biogenic debris contributing to detectable flux anomalies is remote.

In situ microbial production—The third possible source of flux anomalies is midwater microbiological production.

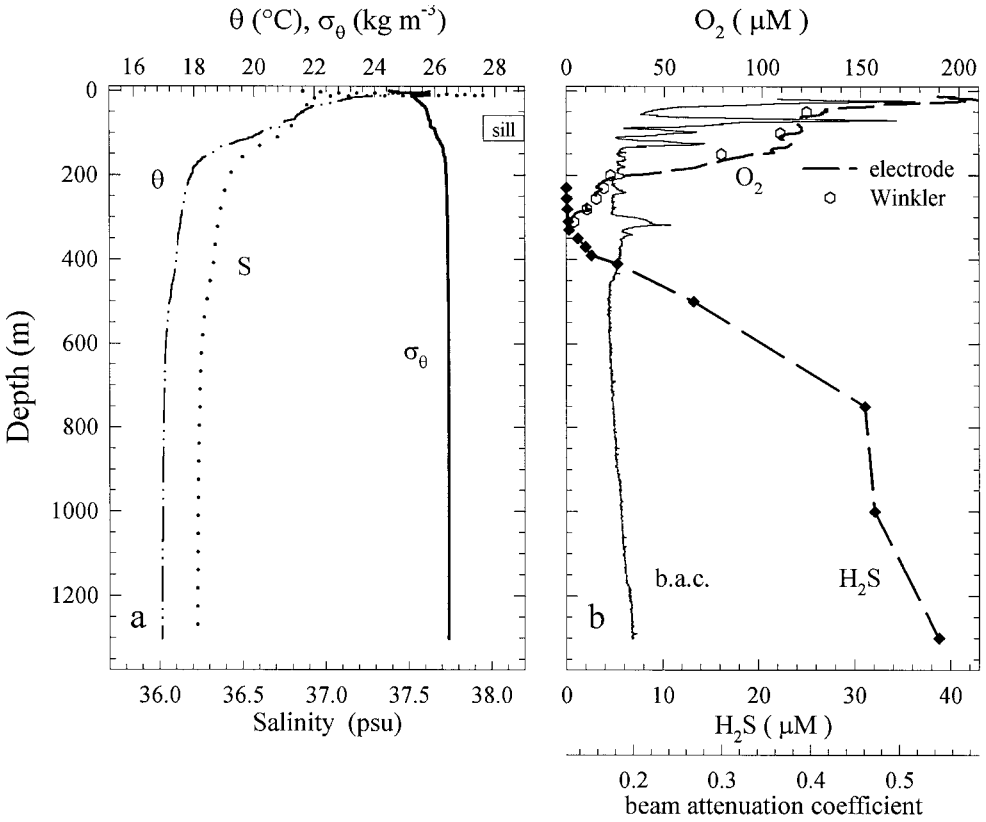

Fig. 3. Typical example of vertical distribution of hydrographic properties at Sta. CARIACO on 7 Jul 98 (CAR-32). (a) Potential temperature, θ, salinity, S, and water density, σ_θ. (b) Dissolved O_2 concentrations, as determined by YSI electrode and Winkler titrations (courtesy of Y. Astor), H_2S concentrations, and particle light scattering, expressed as beam attenuation coefficient relative to clear water.

At least one peak in the transmissometer's beam attenuation, an indication of high particle concentrations, is typically present in the vicinity of the O_2-H_2S interface (*see Fig. 3b*). Given the uniformity of water density in this zone, accumulation of sedimenting particles along weak isopycnals is highly improbable. A more plausible explanation is that discrete particle layers are formed by in situ microbiological activity in response to chemical gradients or formed by transformations of redox-sensitive elements, such as Mn and Fe, which precipitate when oxidized (Tuttle and Jannasch 1973*a;* Nealson and Myers 1992). We have repeatedly observed elevated concentrations of bacteria and high heterotrophic activity at depths where H_2S first appears. During all cruises analyzed to date, maxima in concentrations of not only bacteria, but also VLPs and protozoans (flagellates and ciliates), are observed in the vicinity of the O_2-H_2S interface.

Frequently, as exemplified by observations from CAR-32 (Fig. 4a), two or more peaks in microbial biomass occur immediately above and below the interface. Peaks in microbial biomass below the interface, including bacteria, VLPs and flagellates, correspond to the deep particle maximum detected by the transmissometer (Fig. 3b). Biomass in these secondary peaks is always within in the same order of magnitude as in the primary maximum of the photic zone ($\leq$60 m). Furthermore, these peaks almost always occur at depths between the shallowest two sediment traps (275 and 455 m). This distribution of microbiological biomass argues against the use of strictly one-dimensional vertical models of carbon flux with depth, in which export production from the photic zone is progressively decomposed (Fig. 2a) and becomes increasingly energy depleted and, hence, capable of supporting diminishing biomasses of heterotrophs with depth (Cho and Azam 1988; Karl et al. 1988). Secondary maxima in BNP and acetate uptake have been consistently observed within the RTZ on 11 cruises, residing below a zone of low activity in intermediate waters (100–250 m), as exemplified by CAR-32 (Fig. 4b). Concordant with biomass distributions, heterotrophic activity profiles strongly suggest a midwater source of labile organic matter that supports an active microbial food web, complete with bacterivores and viral pathogens.

Chemoautotrophic production—The preceding observations demonstrate that, unlike the layers immediately above and below, the RTZ maintains a prolific microbial food web. We propose that chemoautotrophic production is the foundation of this food web, providing new labile organic matter. In this zone of the Cariaco Basin and Black Sea, significant rates of dark DIC assimilation and H_2S oxidation have been reported elsewhere (Sorokin 1972; Tuttle and Jannasch

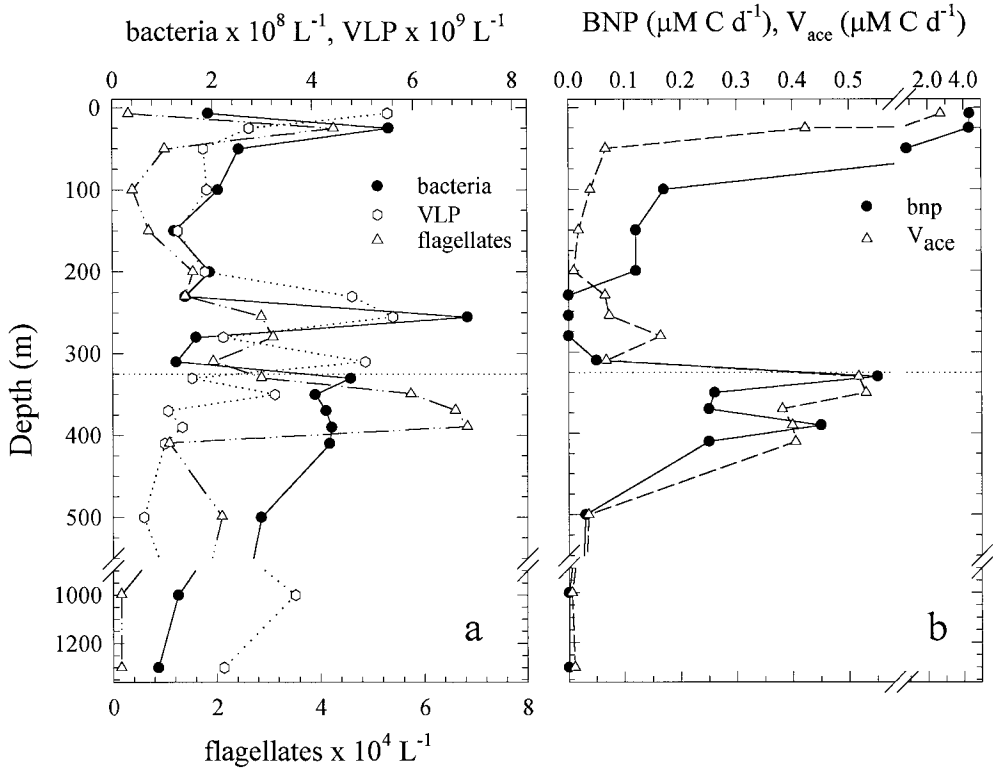

Fig. 4. Typical vertical profiles of microbial abundances and heterotrophic activities from Sta. CARIACO on 7 Jul 98 (CAR-32). (a) Abundances of bacteria, heterotrophic flagellates, and viral-like particles (VLPs). (b) Bacterial net production (BNP) and uptake (incorporation and respiration) rates of acetate, V_{ace}. Horizontal dotted line defines the oxic-anoxic interface in both panels.

1973*a*, 1979; Morris et al. 1985; Jørgensen et al. 1991; Karl and Knauer 1991; Sorokin et al. 1995). In the present study, rates of dark inorganic carbon assimilation (DCA) were measured on seven occasions (9 Nov 96, 8 May 97, 14 Nov 97, 10 Mar 98, 7 Jul 98, 7 Nov 98, and 7 May 99). Peak DCA rates varied by over a factor of six, from 0.4 to 2.5 μM C d^{-1}. During CAR-32, for example, DCA rates reached 2.5 μM C d^{-1} at ~350 m (Fig. 5). Areal rates of DIC assimilation, integrated from 0 to 100 and 310 to 410 m, were 37 and 123 mmol C m^{-2} d^{-1}, respectively, illustrating that, at times, DIC assimilated at depth, presumably by chemoautotrophic bacteria, can exceed by a significant margin DIC that has been fixed photosynthetically. Obviously, such imbalances cannot be sustained indefinitely, because the energy driving this production is derived from reduced products of diagenesis at depth, which is ultimately fueled by primary production. However, current geochemistry of the deep Cariaco Basin reflects accumulation of decompositional products from previous decades and possibly centuries.

In the aphotic zone, DCA can not be ascribed exclusively to chemoautotrophy, because it is the sum of chemoautotrophy and anaplerotic reactions by heterotrophs and can be expressed as, DCA = BAP + *a*BHP, where BAP is bacterial autotrophic production, *a* is proportion of DIC in total heterotrophic C incorporation, and BHP is bacterial heterotrophic production (Karl and Knauer 1991). The proportion of total heterotrophic production supported by DIC assimilation (*a*) through tricarboxylic acid cycle–associated pathways (via phosphoenolpyruvate carboxylase) is growth-dependent, varying from 0.04 to 0.08 over the range of growth rates relevant to this study (Li 1982). If most of the DCA were attributable to heterotrophy, as suggested by Morris et al. (1985) for the Cariaco Basin, then bacterial production within the RTZ during our study would be in the range of 338–1988 mmol C m^{-2} d^{-1} or equivalent to 510%–1,160% of overlying primary production. Such disparities could only be sustained if large amounts of allochthonous organic matter were continuously advected horizontally into the system below 250 m. However, CARIACO sediment-trap samples show little evidence of allochthonous input of organic matter (Thunell et al. 2000). Parallel measurements of heterotrophic BNP during the CARIACO time series demonstrate that, even if $a = 0.08$, the mean *a*BHP only amounts to 2.2% of total DCA. Therefore, we conclude that >97% of DCA below 250 m can reasonably be ascribed to chemoautotrophic bacteria whose metabolism is presumably fueled by upward fluxes of inorganic reductants, such as reduced sulfur species and possibly NH_4^+, from anoxic waters.

Depth distributions and rates of chemoautotrophic activity suggest that this process is central to the microbial ecology of waters below the O_2-H_2S interface. The depth of maximum chemoautotrophic activity occurred 10–30 m below secondary maxima in total bacterial abundance and BNP in all observations (Figs. 4 and 5). However, peaks for microbial abundance and heterotrophic activity always overlapped with those of chemoautotrophic activity. To illustrate che-

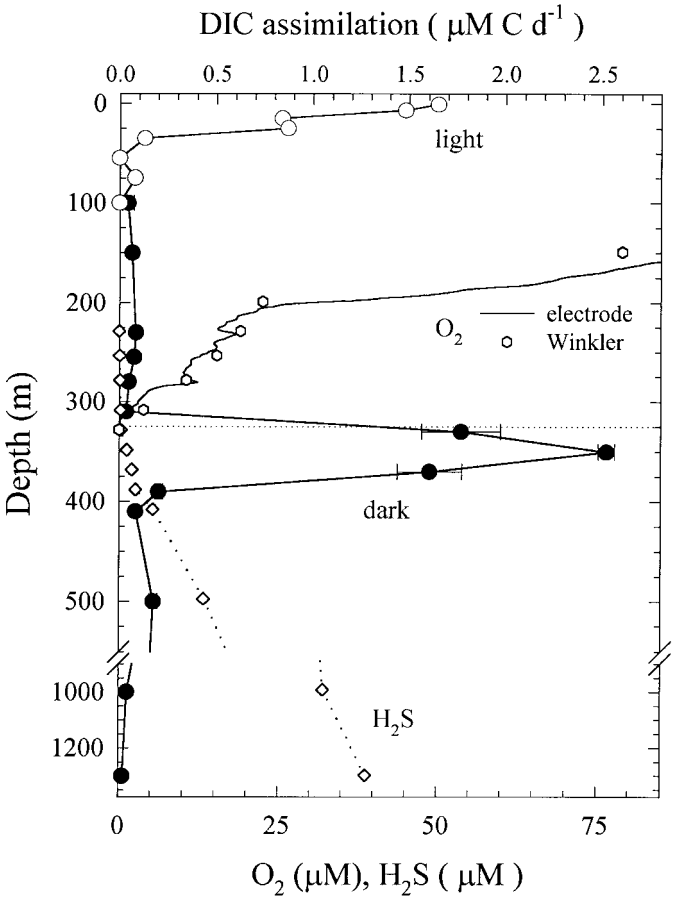

Fig. 5. Example of vertical profiles for contemporaneous measurements of ^{14}C-bicarbonate assimilation rates in light (photosynthesis) and dark (chemoautotrophic + anaplerotic reactions) incubations of samples from Sta. CARIACO. Dark carbon assimilation and primary production were measured on 7 and 9 Jul 98 (CAR-32), respectively. Presented as means of triplicate incubations ±1 SD. In many instances, errors of measurement are no wider than symbols. Profiles of O_2 and H_2S concentrations illustrate redox gradients. Horizontal dotted line defines the oxic-anoxic interface.

moautotrophy's importance in local microbiological production, heterotrophic bacterial production, integrated from 310 to 410 m, only amounted to 2.8, compared with 123.2 mmol C m^{-2} d^{-1} produced autotrophically over the same depth interval in July 1998. Commonly, peaks in chemoautotrophy coincided with those of flagellated protozoans and VLPs (Figs. 4a and 5). Elevated concentrations of predators and pathogens can only be sustained if the prey/hosts are actively growing. Therefore, we infer that chemoautotrophy leads to elevated bacterial production locally, thereby stimulating trophic transfer of newly assimilated DIC to predators and pathogens and to heterotrophic bacterioplankton through mortality processes. The degree of coupling between these processes and secondary vertical export to the sediments is unknown but could be significant.

Temporal variability in DIC assimilation—Although DCA is a persistent feature below the interface, its magnitude and depth distribution varied significantly over time (Fig. 6). Chemoautotrophy is usually defined by a single peak, 60–100 m wide, but on two occasions two peaks were evident, 10 Mar 98 (CAR-29) and 7 Nov 98 (CAR-36). The shallower peak observed during CAR-29 at 255 m may indicate chemoautotrophic activity of nitrifying bacteria (discussed below). The split peak observed during CAR-36 may represent a remnant feature created by lateral intrusions of oxygenated water or by a mixing event. In most cases, the depth of maximum DCA was below the O_2-H_2S interface, which has migrated on the order of 75 m in response to physical forcing during the CARIACO program (Fig. 6).

Carbon assimilation within the chemoautotrophic layer varied between 27 and 159 mmol C m^{-2} d^{-1}, compared with contemporaneous measurements of primary production, varying from 29 to 392 mmol C m^{-2} d^{-1} (Fig. 7). The rates of net primary production varied by a factor of 13.5, which is indicative of strong seasonal differences between upwelling (Jan–May) and nonupwelling (Jun–Dec) seasons. In contrast, rates of chemoautotrophic production only varied by a factor of 5.9 during the same sampling period (Fig. 7). Averaged over the observation period, chemoautotrophic production accounts for 62% of photoautotrophic production; 82 versus 120 mmol C m^{-2} d^{-1}. No statistically significant relationship between surface and midwater autotrophy is apparent from existing data. Nor can we correlate variations in chemoautotrophy with vertical flux anomalies (Fig. 7). The unreasonably high ratio of chemo- to photoautotrophy and the absence of any correlation between these two processes or sedimentation anomalies underscores two facts—the basin is not in steady state (Scranton et al. 1987; Holmen and Rooth 1990; Zhang and Millero 1993), and our temporal sampling was insufficient to fully resolve amplitudes of variances in both forms of production. We also note that observed intrusions of water (Fig. 7) do not correlate with vertical flux anomalies. Therefore, horizontal advection below sill depth does not appear to directly contribute to the enrichments in particles observed in the RTZ. Furthermore, effects of upwelling and its relaxation on chemoautotrophy are not evident from our observations.

Our chemoautotrophic production estimates were equivalent to between 10% and 333% of contemporaneous measurements of primary production, compared with 10%–32% in the Black Sea in 1988 (Jørgensen et al. 1991; Karl and Knauer 1991) and 17%–58% in the Cariaco in 1973 (Tuttle and Jannasch 1979). However, simultaneous measurements of primary production were performed only by Karl and Knauer (1991) and Jørgensen et al. (1991), whereas Tuttle and Jannasch (1979) and Sorokin et al. (1995) based their comparisons on historical measurements and indirect estimates that do not account for temporal variability.

We expect that maximum rates of chemoautotrophy would be higher than those reported by Tuttle and Jannasch (1979) (0.23 μM C d^{-1} or 11–18 mmol C m^{-2} d^{-1}), because H_2S concentrations in Cariaco bottom waters, which presumably fuel chemoautotrophic processes, were lower in 1973 than they are at present (30 vs. $\leq$ 76 μM). Furthermore, although present and past Cariaco studies share the same experimental protocols, we used 0.22-μm filters rather than the 0.45-μm filters used by Tuttle and Jannasch (1979) to assay assimilated ^{14}C. The 0.45-μm filters probably captured fewer la-

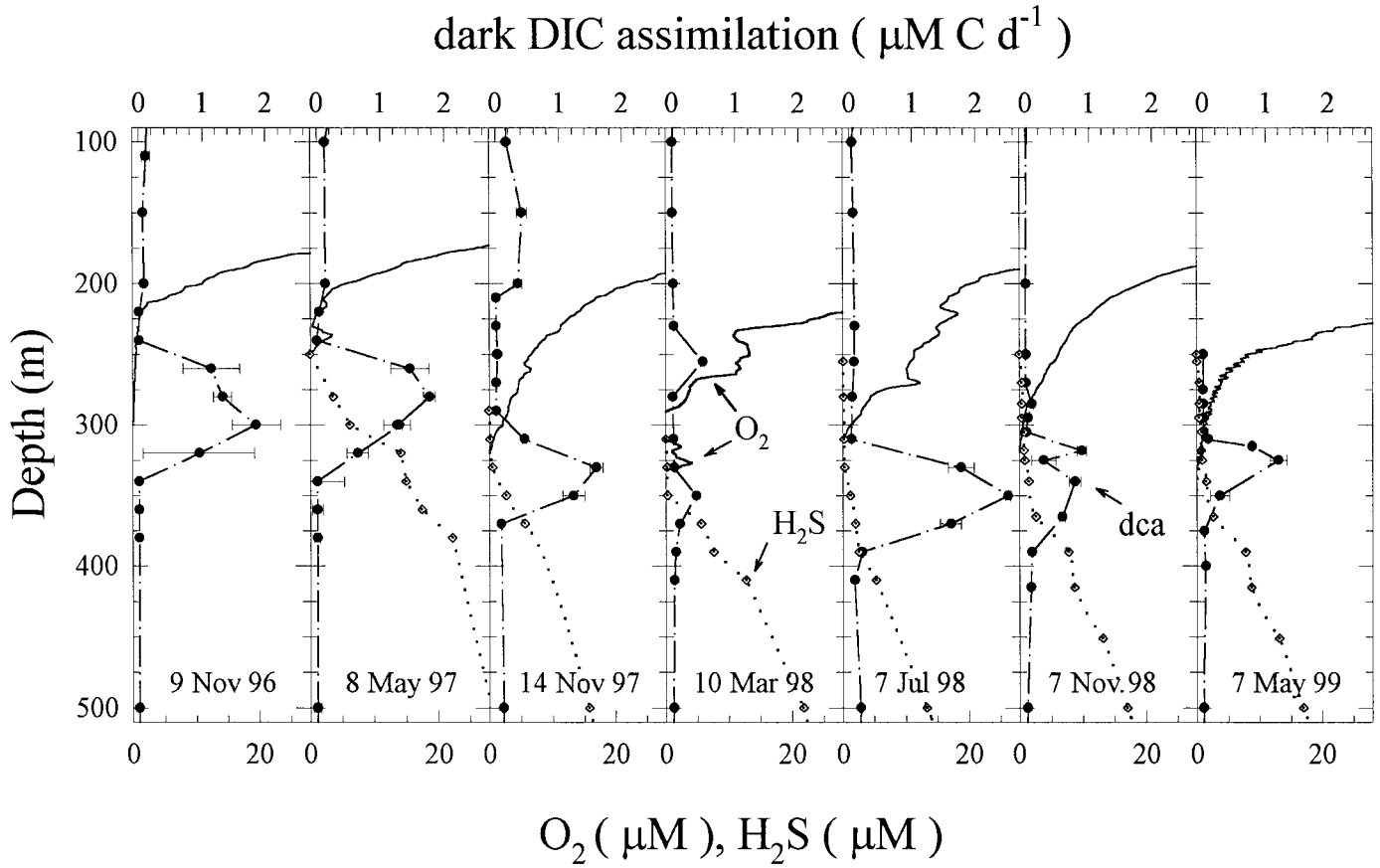

Fig. 6. Temporal variability of dark DIC assimilation (dca) rates in the chemoautotrophic layer and distributions of O_2 (electrode data) and H_2S (discrete samples) at Sta. CARIACO. Mean and 1 SD of triplicate incubations presented for dark DIC assimilation. In many instances, errors of measurement are no wider than symbols. H_2S data not available for CAR-13. 9 Nov 96 = CAR-13, 8 May 97 = CAR-19, 14 Nov 97 = CAR-25, 10 Mar 98 = CAR-29, 7 Jul 98 = CAR-32, 7 Nov 98 = CAR-36, and 7 May 99 = CAR-42.

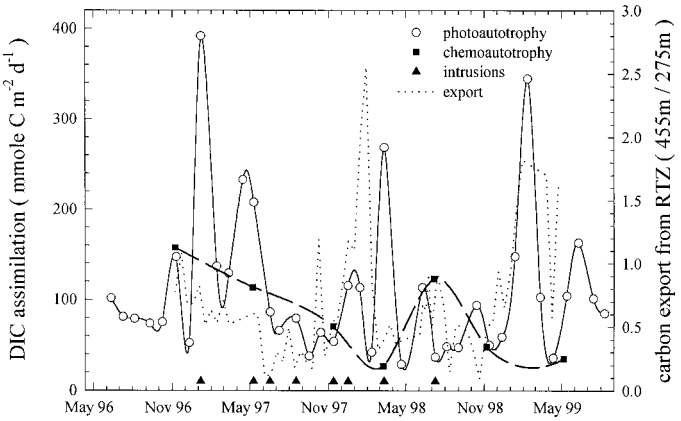

Fig. 7. Temporal variability in areal net primary and chemoautotrophic production and carbon export from RTZ at Sta. CARIACO from Jun 1996 to May 1999. Production values represent ^{14}C-incorporation into >0.22 μm particles in light and dark incubations, integrated over eight depths in the upper 100 m (photic zone usually $\leq$55 m) and over 5–7 depths in the upper 60–100 m of the RTZ. Carbon export from RTZ expressed as organic carbon fluxes to 455 m normalized to contemporaneous measurements at 275 m. Values $\geq$1 are considered to be flux anomalies. Solid triangles are dates of known midwater intrusions over the sill (Y. Astor pers. comm.).

beled cells. It is also possible that they did not sample the zone of highest activity because of the lower vertical resolution of their sampling.

Comparisons to the Black Sea—Maximum chemoautotrophic rates for the Black Sea (0.32–1.50 μM C d^{-1}) are similar to those we measured in the Cariaco Basin (Jørgensen et al. 1991; Karl and Knauer 1991; Sorokin et al. 1995), with the lowest rates being reported for the 1988 expedition, when a wide suboxic zone (O_2- and H_2S-free) replaced an interface where O_2 and H_2S overlap. Integrated chemoautotrophic production reported for the Black Sea varied from 2 to 27 mmol C m^{-2} d^{-1}, depending on the station and depth intervals included (14–90 m).

Lower areal chemoautotrophic production rates are expected for the Black Sea than for the Cariaco. Even though bottom H_2S concentrations in the Black Sea are ~7 times greater than those in the Cariaco Basin (Table 1), the zone of dark DIC assimilation is usually $\leq$40 m thick in the former, compared with 60–100 m thick in the Cariaco Basin. The relative narrowness of the RTZ in the Black Sea (45–95 m in 1988) and steepness in the H_2S gradients results from a much higher degree of thermohaline stratification. Sulfide gradients in the Black Sea are 5–6 times steeper, and

Table 1. Comparison of variables relevant to chemoautotrophy in the Black Sea and Cariaco Basin.*

Variable	Black Sea	Cariaco Basin
Maximum C-assimilation rate (μM C d^{-1})	0.32–1.50	0.40–2.52
Chemoautotrophic zone (m)	14–90	80–100
Areal C-assimilation rate (mmol C m^{-2} d^{-1})	2–27	26–157
Maximum H_2S concentration (μM)	~550†	76‡
H_2S gradient (μM m^{-1})	0.51–0.83	0.08–0.18
Density gradient ($\Delta\sigma_\theta$ m^{-1} $\times 10^3$)	8.80–13.30	0.15–0.32
Diffusive H_2S flux§ (mmol m^{-2} d^{-1})	0.61–0.99	0.71–1.35
H_2S required‖ (mmol m^{-2} d^{-1})	14–189	186–1101
O_2 flux§ (mmol m^{-2} d^{-1})	ND¶	1.11–3.87
O_2 required# (mmol m^{-2} d^{-1})	2–27	26–157
NO_3 flux§ (mmol m^{-2} d^{-1})	0.12–0.28††	0.37–1.59
NO_3 required‡‡ (mmol m^{-2} d^{-1})	22–302	297–1762

* Unless otherwise noted, data from Jørgensen et al. (1991) and Sorokin et al. (1995) were used for the Black Sea, and data from this study were used for the Cariaco Basin.
† Karl (1978).
‡ Scranton et al. (in press).
§ Flux estimated as product of concentration gradient and vertical eddy diffusivity, $F = K_i \Delta C/\Delta z$ (Scranton et al. 1987).
‖ Assumes yield of 0.14 mol CO_2 assimilated per mol H_2S oxidized based on field observations of Jørgensen et al. (1991) and laboratory studies of Kelly (1989).
¶ ND, not determined.
# Theoretical stoichiometry of $1C:1O_2$ for aerobic chemoautotrophy.
†† Estimated from Murray et al. (1995).
‡‡ Assumes 1.6 mol NO_3 required for complete oxidation of H_2S to SO_4^{2-} through dissimilatory denitrification, $2\ NO_3^- + 10e^- \rightarrow N_2$.

water density gradients (8.8–13.3 $\Delta\sigma_\theta$ m^{-1} $\times$ 10^3) are 42–59 times greater than comparable zones in the Cariaco Basin (Table 1). Density gradients calculated between 200 and 500 m at Sta. CARIACO only varied from 0.15 to 0.32 $\Delta\sigma_\theta$ m^{-1} ($\times 10^3$), indicative of comparatively subtle changes in water density (Table 1). The relative physical homogeneity of the Cariaco's anoxic zone presents a considerably smaller barrier to vertical diffusive and mixing processes. Therefore, higher fluxes of H_2S and O_2 to the interface, and broader features are expected for the Cariaco Basin. If vertical eddy diffusivity, K_z, were the only operative transport process and the sediments (>1 km from interface) are the primary H_2S source (Scranton et al. 1987), then H_2S fluxes estimated as the product of the concentration gradient and K_z would be slightly higher for the Cariaco Basin than it is for the Black Sea; 0.71–1.35 and 0.61–0.99 mmol m^{-2} d^{-1}, respectively (Table 1). Trends in H_2S diffusive fluxes in both basins are consistent with observed rates of DIC assimilation. However, diffusive fluxes alone are insufficient to explain observations from either basin (Murray et al. 1995; this study).

Flux balance conundrum—In order for chemical redox reactions to proceed, the supply of electron donors must balance that of electron acceptors. In the Cariaco Basin, however, we have been unable to balance fluxes of electron donors and acceptors (H_2S, O_2, and NO_3^-) in electron equivalents with DIC assimilation rates on the basis of observed chemical gradients, estimates of vertical eddy diffusion, and typical stoichiometries. Measured rates of DIC assimilation surpass estimated rates of supply of electron donors and acceptors to the RTZ by a significant margin. This has been the case for studies of a number of other anoxic basins (Tuttle and Jannasch 1979; Jørgensen et al. 1991; Murray et al. 1995). Jørgensen et al. (1991) suggested that 7–9 mol of H_2S are required for mixed chemoautotrophic communities to assimilate 1 mol of CO_2 under field conditions. Culture studies with several species of marine sulfide oxidizers report that between 2.4 and 7.2 mol of H_2S are required to assimilate 1 mol of CO_2 by chemolithotrophy, with demand for reductant depending on species and environmental conditions (Tuttle and Jannasch 1977; Kelly 1989). When a molar requirement of 7 is used, fluxes of H_2S into the Cariaco's RTZ only account for between 0.1%–0.4% of the chemoautotrophic demand (Table 1). The flux imbalance is only slightly smaller in the Black Sea, where the supply of H_2S accounts for 0.5%–4.4% of the demand. Even under the assumption of the theoretical maximum efficiency (where stoichiometry is $1H_2S:1CO_2:1O_2$ for aerobic sulfide oxidation), the Cariaco's flux balance is improved only minimally, with diffusive H_2S fluxes accounting for 0.7%–2.8% of the demand for reductant.

Aerobic sulfide oxidation is constrained by low O_2 diffusive fluxes to the interface, potentially accounting for 2.5%–4.3% of estimated demand in the Cariaco Basin (Table 1). Nitrate can also serve as an electron acceptor for denitrifying sulfide oxidizers, like *Thiomicrospora denitrificans*, *Thioploca* spp. or *Beggiatoa* spp. (McHatton et al. 1996; Jorgensen and Gallardo 1999) and typically penetrates deeper in the water column than O_2. However, eddy diffusion can only deliver on the order of 0.4–1.6 mmol NO_3^- m^{-2} d^{-1} to the interface, satisfying an additional 0.1% of the estimated demand for oxidant (Table 1). Even under the assumption of reduction of NO_3^- to NH_4^+, as has been suggested for *Thioploca* communities (Farias 1998), and ideal stoichiometry, $1H_2S:1CO_2:1NO_3$, diffusive fluxes of NO_3^- could only supply 1.0%–1.4% of the demand for oxidant. Similar analyses for the Black Sea suggest that NO_3^- supplies 0.1%–0.6% of the oxidant demand by diffusion (Table 1). Clearly, diffusive fluxes of electron donors and acceptors are insufficient to balance observed productivity across the interface in either anoxic basin.

Advective processes—One problem with the preceding estimates is that horizontal and vertical advective processes in the Cariaco Basin are ignored, mainly because they are so poorly understood. Although lateral intrusions are probably not energetic enough to import biogenic debris into the central basin as discussed above, they do import dissolved (e.g., O_2), and probably colloidal, materials. O_2 anomalies, which are indicative of lateral intrusions, were detected between 225 and 330 m on 8 May 97, 14 Nov 97, 10 Mar 98, and 7 Jul 98, four dates when dark carbon fixation was measured (Fig. 6). Horizontal advection would deliver O_2 to the interface at much higher rates than vertical eddy diffusion. Unfortunately, the magnitude, frequency, and velocity of these intrusions is unknown, because appropriate physical measurements are lacking. The fact that O_2 remains after the water transits from the margin to the eastern basin's center,

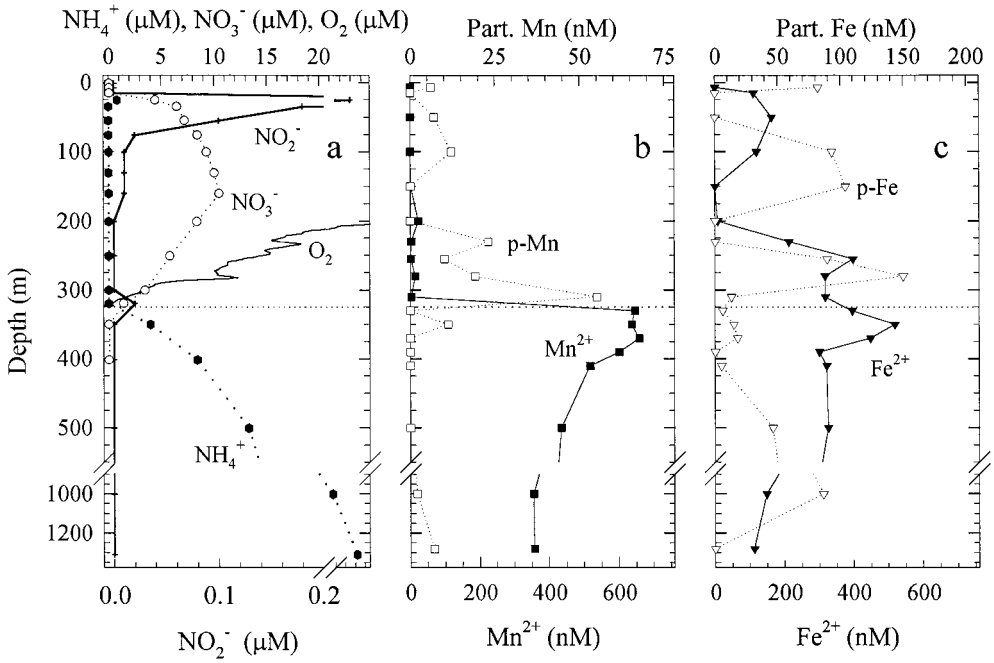

Fig. 8. Vertical profiles of oxidized and reduced forms of nitrogen (a), manganese (b), and iron (c) at Sta. CARIACO on 7 Jul 98 (CAR-32). Although undetermined experimentally, the redox state of particulate Mn and Fe (p-Mn and p-Fe) is assumed to be mostly oxidized above the interface and dissolved to be mostly reduced. Dotted line represents oxic-anoxic interface in all panels.

despite oxygen utilization by chemical and biological reactions in transit, argues that lateral advective fluxes could be relatively large. Whether advective O_2 fluxes are sufficient to balance requirements for oxidant remains to be demonstrated and will depend on the relative demands of aerobic heterotrophy, nitrification, abiotic oxidation of redox-sensitive elements (S, Mn, and Fe), and chemoautotrophy.

The ultimate energy source (e^- donor) for observed chemoautotrophy most likely is H_2S, whether by direct utilization or through intermediate oxidation products (S^0, $S_2O_3^{2-}$, or SO_3^{2-}) not measured in this study. As demonstrated above, vertical diffusive fluxes of H_2S are insufficient to meet demand, so advective processes or intensive cycling of redox pairs must play an important role in meeting this demand, as has been suggested for the Black Sea (Lewis and Landing 1991; Murray et al. 1995). Density (σ_θ) profiles and T-S diagrams clearly illustrate that water in the basin is relatively homogenous below 200 m (Fig 3a). Therefore, only minimal energy needs to be applied to induce vertical mixing. Mechanisms that would provide that energy, however, are not immediately obvious. The surface mixed layer and pycnocline are quite shallow, usually <50 and 120 m, respectively, so wind forcing at the interface is unlikely. Sporadic turbidity flows created by mass wasting of unstable sediments on the basin's walls would cause mixing and upward displacement of water. This process can be catalyzed by seismic activity (Thunell et al. 1999) but may also be induced by other processes, such as gravity flows when gravitational forces exceed the cohesive forces of sediments collecting on inclined surfaces. Lateral intrusions above the O_2-H_2S interface could contribute to deep convective circulation driven by interfacial stress. This could entrain deeper sulfidic waters from the basin's leading margin and advect it into the basin's interior beneath the interface. Such intrusions could also contribute to shear stress–driven vertical mixing. Both processes (advection and mixing) associated with intrusions could deliver H_2S to the interface far more rapidly than background eddy diffusion. The frequency and areal extent of these advective events is unknown. A more elaborate evaluation of mixing terms is premature, given existing data.

Elemental cycling—One of the surprising features of the chemoautotrophic activity in the Cariaco Basin is that the vast majority of DCA occurs in regions lacking detectable O_2, which implies that bacterial populations use terminal electron acceptors other than O_2. Previous field observations suggest that chemoautotrophy in the absence of O_2 is common in stratified anoxic basins, such as the Cariaco Basin and the Black Sea (Tuttle and Jannasch 1973*a*, 1979; Jørgensen et al. 1991; Karl and Knauer 1991; Sorokin et al. 1995). In our study, peak chemoautotrophic production coincided with the disappearance of NO_3^- and particulate Mn and Fe, as well as enrichments of dissolved Mn^{2+} and Fe^{2+} (Fig. 8). These distributions suggest that denitrifying and metal-reducing sulfide-oxidizing populations may be important in DIC assimilation. Anaerobic chemoautotrophs that oxidize H_2S and $S_2O_3^{2-}$ at the expense of NO_3^- have been isolated from the Cariaco Basin, the Black Sea, and suboxic mat communities dominated by *Beggiatoa*, *Thioploca*, or *Thiomargarita* spp. in upwelling regions (Tuttle and Jannasch 1973*a;* McHatton et al. 1996; Jørgensen and Gallardo 1999; Schulz et al. 1999). As described above, the estimated

fluxes of NO_3^- appear to be insufficient to support high rates of denitrification below the O_2-H_2S interface.

Distributions of NH_4^+, NO_2^-, and NO_3^- suggest that dissimilatory nitrogen cycling is consistently operative in two layers in the Cariaco Basin (Fig. 8a) and may account for some portion of observed chemoautotrophy. In all profiles analyzed ($n = 5$), nitrogen speciation suggests that nitrifying bacteria are active between 25 and 75 m, where slight enhancements of DCA are also evident (not presented). The shallow NO_2^- peak (Fig. 8a) may be indicative of NH_4^+ oxidation in this layer, where NH_4^+ concentrations generally varied from 0.1 to 0.7 μM. Subsequent in situ oxidation of NO_2^- contributes to NO_3^- accumulation in waters from 25 to 200 m, and phytoplankton assimilation accounts for total depletion in shallower waters. A nitrifying community may also reside slightly above the O_2-H_2S interface, where trace concentrations of O_2 are available and a strong NH_4^+ gradient exists. For example, on 10 Mar 98 the upper peak in DCA at 255 m (Fig. 6) corresponds to a peak in NO_2^- (0.1 μM) and NH_4^+, NO_3^-, and O_2 concentrations of 0.2, 4.5, and $\sim$10 μM, respectively, whereas the deeper peak occurred in sulfidic waters devoid of NO_2^-, NO_3^-, or O_2 (only O_2 data are presented). Nitrifiers are expected only in sulfide-free zones, because these strictly aerobic chemoautotrophs are strongly inhibited by H_2S (B. B. Ward pers. comm.).

The sinking of particulate oxidants across the interface may be an alternative transport mechanism to eddy diffusion and lateral advection. Although concentrations are relatively low (10–600 nM), distributions of Mn and Fe are consistent with the hypothesis that these metals serve as ''redox shuttles.'' As applied to metal-respiring heterotrophs in the Black Sea by Nealson and Myers (1992), dissolved and reduced metals diffuse up to oxic waters and rapidly oxidize, abiotically forming colloids and particulates (oxides and oxyhydroxides). Newly-formed particulates sink back into the RTZ and are biologically reduced through dissimilatory respiration by bacteria, then diffuse back up to the interface for reoxidation. This mechanism permits deeper and faster penetration of oxidant into the RTZ and repetitive cycling of the same redox pairs. For example, particulate phases of Mn and Fe in the Cariaco Basin are most abundant in oxic waters, especially just above the interface (Fig. 8b,c). Particulate metals that occur in anoxic waters are most likely metal-sulfide precipitates, carbonates, or detrital phases (Lewis and Landing 1991). Dissolved Mn^{2+} and Fe^{2+} phases, which may also include colloidal metal sulfides (Lewis and Landing 1991) or oxides, are enriched in the upper 75 m of the anoxic zone, coincident with peaks in chemoautotrophic activity and bacterial abundances (Figs. 4, 5). These distributions suggest a zone of intensive reduction of metal oxides just below the interface, which must be supplied from above. On 7 July 98, the particulate Fe and one of the particulate Mn peaks coincided with O_2 anomalies and may indicate either lateral injection of colloidal metals or in situ oxidation of metals (Fig. 8). The deeper particulate Mn peak could be a remnant of an earlier intrusion in which the O_2 has been depleted below detection limits by any number of oxidative processes. Thus, supply of metal oxides to the interface may be controlled by both lateral intrusion and settling of metal oxide particles, both of which are likely to be orders of magnitude faster than diffusive processes.

Jørgensen et al. (1991) speculated, on the basis of distribution profiles, that anaerobic chemoautotrophs oxidize H_2S or $S_2O_3^{2-}$ at the expense of oxidized Mn and Fe in the Black Sea. Nealson and Myers (1992) have isolated a heterotrophic bacterium, *Shewanella putrefaciens*, capable of reducing metal oxides at the expense of several simple organic substrates. A chemoautotrophic bacterium, capable of disproportionating S^0 or $S_2O_3^{2-}$ and dependent on extracellular ferrihydrite [$Fe(OH)_3$] to scavenge reactive H_2S, has recently been isolated from anoxic sediments (Finster et al. 1998). Although freshwater species have been isolated (Pronk et al. 1992), we are unaware of any reports documenting facultative or obligate anaerobes capable of directly reducing oxidized Mn and Fe at the expense of H_2S, S^0, or $S_2O_3^{2-}$ while supporting chemoautotrophic growth under marine conditions. Considering both thermodynamic and kinetic arguments, Mn oxide, and, to a lesser extent, Fe oxide, should be almost as energetically favorable as NO_3^- in both the Cariaco Basin and the Black Sea (Nealson and Myers 1992).

Amendment experiments were performed to assess the roles of selected electron donors and acceptors in chemoautotrophic metabolism. On two occasions, experiments were conducted with samples collected from six to seven depths, spanning the RTZ. In addition to ^{14}C-bicarbonate, replicate samples were spiked with relatively high concentrations of alternative electron donors or acceptors (N_2-purged), then incubated and processed in the same way as standard samples. DCA by suboxic and anoxic communities was stimulated by amendments of $S_2O_3^{2-}$ in six of nine observations by factors of 1.2–16.4 over parallel unamended samples (Table 2). Stimulation by S^0 amendments was unconvincing, showing slight stimulation ($\times$1.6) at one suboxic depth only (295 m). Depending on redox conditions, $S_2O_3^{2-}$ and S^0 may be used as either reductants or oxidants or, in some cases, as both, through disproportionation (Finster et al. 1998). Results may have been influenced by the low bioavailability of the crystalline S^0 used, or our sample depths may have omitted a narrow layer where S^0 turnover is important, as has been suggested by Hastings and Emerson's (1988) data.

Stimulation of DCA by addition of NH_4^+ was detected in only two of eight observations and was equivocal (1.1–1.8$\times$) at best, suggesting that NH_4^+ provides very little of the reducing power for DIC assimilation in this zone. However, NH_4^+ may have stimulated activity at depths shallower than those tested. In deeper anoxic samples, equimolar MnO_2 and Fe_2O_3 amendments stimulated DIC assimilation rates by factors of 1.1–4.3 and 1.9–8.5, respectively while having no effect on the shallower samples, perhaps because of trace ambient concentrations of NO_3^- or O_2. In deeper samples, addition of NO_3^- and air (deliberate headspace) stimulated DIC assimilation rates by factors of 1.5–15.7 and 6.7 over unamended samples, respectively (Table 2). These results suggest that chemoautotrophic communities are dominated by bacteria capable of oxidizing thiosulfate, and presumably sulfide, and capable of facultative respiration of metals and NO_3^- (denitrifiers). In agreement with earlier observations from the Cariaco Basin and the Black Sea (Tuttle and Jan-

Table 2. Stimulation of dark DI^{14}C assimilation by amendments with alternate electron donors or acceptors. Other than amendments, experimental conditions same as described in Materials and Methods. Results are expressed as ratio of ^{14}C-bicarbonate biologically fixed in amended samples to that fixed in parallel unamended samples. Ranges relative to mean are presented in parentheses for treatments that were duplicated. Depth ranges of dark DIC assimilation: 7 Jul 98 = 320–390 m, peak = 350 m; 7 Nov 98 = 310–390 m, peak = 318 m.

Date	Depth (m)	S_2O_3 (50 μM*)	Range (m)	S^0†	NH_4^+ (50 μM)	Range (m)	MnO_2 (9.0 μM)	Range (m)	Fe_2O_3 (4.5 μM)	Range (m)	NO_3^- (50 μM)	Air (5 ml)
7 Jul 98												
Suboxic	310	1.25	(0.76)	ND	1.00	(0.00)	ND†		ND		ND	ND
	330	2.03	(0.20)	ND	1.11	(0.09)	ND		ND		ND	ND
Anoxic	350	1.94	(0.08)	ND	1.00	(0.00)	1.11	(0.11)	1.00	(0.00)	ND	ND
	370	ND‡		ND	ND		1.00	(0.00)	1.00	(0.00)	ND	ND
	390	ND		ND	ND		1.00	(0.00)	1.00	(0.00)	ND	ND
	410	ND		ND	ND		1.34	(0.07)	1.00	(0.00)	ND	ND
7 Nov 98												
Suboxic	270	1.00		1.00	ND		ND		ND		1.00	ND
	285	1.00		1.00	1.00		1.00		1.00		1.00	ND
	295	1.00		1.60	1.00		1.00		1.00		1.50	ND
	340	ND		1.00	ND		ND		ND		ND	ND
Anoxic	365	1.60		1.00	1.00		1.00		1.00		1.00	ND
	390	15.40		ND	1.80		3.10		8.50		15.70	ND
	415	16.40		1.00	1.00		4.30		1.90		ND	6.70

* Final concentrations in samples.
† Saturated suspension.
‡ ND, not determined.

nasch 1979; Sorokin et al. 1995), nitrifiers do not appear to be responsible for much DIC reduction near the oxic-anoxic interface.

Our stimulation experiments verify only the metabolic potential of bacterial communities and provide no indication of in situ rates of various redox reactions. However, these results are consistent with selective enrichment cultures derived from the RTZ during this study, in which autotrophic $H_2S/S_2O_3^{2-}$-oxidizing denitrifiers, $S_2O_3^{2-}$-oxidizing/Mn^{4+} reducers, S^0 disproportionaters, and S^0 reducers were obtained (Madrid 2000). In our stimulation experiments, one could argue that MnO_2 and Fe_2O_3 oxidized H_2S to S^0 abiotically and thereby stimulated S^0-disproportionating bacteria, rather than metal-respiring bacteria. However, Madrid (2000) demonstrated that cultures from the RTZ reduced MnO_2 autotrophically at the expense of $S_2O_3^{2-}$ in a mineral medium, whereas $S_2O_3^{2-}$ did not reduce MnO_2 abiotically under experimental conditions. This supports the hypothesis that metal-reducing chemoautotrophs are indigenous to the RTZ. The importance of autotrophic $H_2S/S_2O_3^{2-}$-oxidizing denitrifiers below the interface is further underscored by the frequency of their occurrence in 16S rDNA clonal libraries established from polymerase chain reaction (PCR)–amplified nucleic acids (Madrid 2000). It appears that this physiological group dominated the RTZ, if, in fact, PCR amplified genomes in proportion to their natural abundances.

A variety of physiological types of chemoautotrophic and heterotrophic bacteria probably inhabit the region immediately above and within the RTZ, many coexisting syntrophically (Tuttle and Jannasch 1973*b*). Fermentative bacteria appear, on the basis of H_2 profiles (Scranton et al. 1984), enrichment cultures, and 16S rDNA libraries (Madrid 2000), to be particularly important just below the interface and throughout the anoxic zone. Sulfate-reducing bacteria associated with anoxic microenvironments above the interface may also be important, as suggested by Hastings and Emerson (1988). Heterotrophs, which require organics for growth but also assimilate CO_2 at the expense of $S_2O_3^{2-}$, may play a role in energy flow and sulfur cycling as well (Tuttle and Jannasch 1977). Furthermore, facultative anaerobes capable of reducing sulfite, thiosulfate, and tetrathionite to sulfide and thiosulfate at the expense of low-molecular-weight fatty acids may also be present (Tuttle and Jannasch 1973*b*). Sulfur cycling may be very intensive within the RTZ, with chemolithotrophy facilitated by sequential oxidations and reductions of sulfur species by different physiological groups, and demands closer examination in the future.

Symbiotic associations—The above discussion assumes that all motion of chemicals and bacteria is controlled by advection or diffusion. Participation of migratory organisms is also possible. Migration of free-swimming chemoautotrophic bacteria across chemical gradients to sequester electron acceptors, analogous to acquisition of NO_3^- by *Beggiatoa* spp., *Thioploca* spp., or *Thiomargarita namibiensis* (McHatton et al. 1996; Jørgensen and Gallardo 1999; Schulz et al. 1999), seems unlikely, because the small, free-living bacteria in this system are theoretically only capable of migration rates on the order of 0.15 m h^{-1} (Khan 1990). They would not be able to span distances (≥30 m) between the productivity maximum and detectable NO_3^- in less than 8 d, which is energetically unfeasible. However, symbiotic associations with larger, faster-swimming organisms, such as protozoa, could provide a possible adaptive strategy to circumvent this problem. Anaerobic ciliates have been documented to migrate along O_2 and light gradients at rates of up to 5 m h^{-1} (Finlay et al. 1987), which is sufficiently fast

to complete a round trip in the Cariaco in 12 h. Significant populations of anaerobic protozoa have been reported for the Black Sea, anoxic regions of the Baltic Sea, and a variety of smaller bodies of marine and freshwater (Fenchel et al. 1990; Setälä 1991; Zubkov et al. 1992). Bird and Karl (1991) reported a peak the abundance for a symbiont-bearing ciliate centered at the depth at which H_2S first appears in the Black Sea.

Some anaerobic species of phagotrophic ciliates lack mitochondria and are known to ferment substrates derived from prey, supplying volatile fatty acids and H_2 to endosymbiotic methanogens and ectosymbiotic sulfate-reducers (Fenchel et al. 1990). However, results from our amendment experiments (Table 2), methane distributions (Scranton 1988), and the absence of Archaea in the 16S rDNA library compiled from this layer (Madrid 2000) suggest that methanogens (symbiotic or free-living) are not major consumers of DIC in the Cariaco's RTZ. Anaerobic protozoa are known to form symbiotic associations with algae (zoochlorellae) and purple sulfur bacteria (Fenchel and Finlay 1994). Perhaps undescribed symbiotic associations of chemoautotrophic bacteria, capable of sequestering NO_3^-, like the free-living *T. namibiensis* (Schulz et al. 1999), occur with migratory ciliates. We have repeatedly observed maxima in abundances of ciliates and flagellates within the Cariaco's RTZ (unpubl. data). The presence of symbionts in some of these protozoa is expected but needs to be confirmed. Such an association would help explain how anaerobic sulfide oxidizers could acquire sufficient oxidant from shallower horizons and return to sulfidic waters to oxidize sulfur species and assimilate DIC.

Larger-scale implications—Observed cruise-to-cruise variations in rates of chemoautotrophy do not correlate with changes in H_2S and O_2 gradients or with primary production. In fact, midwater carbon production appears to be either decoupled from surface processes or is responsive over much longer timescales than our record. H_2S concentrations have varied significantly over the latter half of this century, with a steady increase until a dramatic drop in 1997 (Scranton et al. 1987, in press; Zhang and Millero 1993). Current levels of chemoautotrophic activity appear to be driven by upward fluxes of H_2S. These fluxes are controlled by organic carbon delivery to the seabed, diagenetic processes within the sediments, externally-forced vertical and horizontal advection, and upward diffusion. Processes contributing to basin ventilation vary over decadal timescales in response to fluctuations in regional wind stress (Holmen and Rooth 1990). Therefore, current chemoautotrophic production may reflect return of stored energy derived from surface export production from the recent past (seasonal, or annual to decadal timescales) and, in essence, represents the basin's memory. The delay between the delivery of fresh particulate organic matter to the basin's interior and arrival of dissolved chemical reductant derived from its remineralization at the interface is not known, nor are the processes that control them fully described.

Werne et al. (2000) used $\delta^{13}C$ and $\delta^{15}N$ signatures in their interpretation of the Cariaco's varved sediments to reconstruct paleoclimate in the tropical western Atlantic region. Their interpretation employs a model based on open ocean oxygenated water in which phytoplankton typically have $\delta^{13}C$ of -8‰ to -24‰ (Ruby et al. 1987; Fry et al. 1991). On the basis of departures in $\delta^{13}C$ and $\delta^{15}N$ from expectations in the varves, they draw inferences about DIC limitation and upwelling intensity. An alternative explanation is possible if a significant portion of sedimentary organic matter is derived from midwater production by chemoautotrophs. It is known that chemoautotrophic bacteria fractionate stable isotopes to a higher degree than photoautotrophs and produce biomass depleted in ^{13}C, ^{15}N, and ^{34}S, resulting in isotopically light biomass (Fry et al. 1991). For example, two species of mesophilic, aerobic, chemolithotrophic bacteria have been found to be ~25‰ depleted in ^{13}C relative to their medium (Ruby et al. 1987). Consequently, their $\delta^{13}C$ could be -26‰ or lower in the Cariaco, where the DIC is -1‰ to -2‰ (Fry et al. 1991). Preuß et al. (1989) have reported even greater ^{13}C fractionation for non–sulfur-oxidizing autotrophic bacteria ($\Delta\delta^{13}C$ of -26.1‰ to -39.7‰, depending on the DIC fixation pathway). Although isotopic signatures of anaerobic, chemolithotrophic bacteria are unknown, it seems likely that chemoautotrophic production in the RTZ will be isotopically light and that the isotopic signature of bacterivorous organisms will be even more depleted in ^{13}C and ^{15}N (Ruby et al. 1987).

If the RTZ has a significant export flux with a distinctively light isotopic signature, which varies out of phase with surface production, then bulk fluxes and isotopic composition of the sediment record may not directly reflect epipelagic and atmospheric processes. Current models interpret isotopically light organic matter (relatively negative) in the sedimentary record as reflecting a period when surface waters were nutrient replete and photoautotrophy was rapid (Werne 2000). Our data suggest that, instead, these may be periods when flux of chemoautotrophically derived material was quantitatively more important. If true, then current paleoceanographic and paleoclimatological interpretations of the Cariaco Basin's sediment record may require reevaluation.

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