Fe-catalyzed sulfide oxidation in hydrothermal plumes is a source of reactive oxygen species to the ocean

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The significance of the Fe-catalyzed oxidation of sulfide by dioxygen in hydrothermal plumes is shown to be a source of reactive oxygen species (ROS) to the deep ocean. ROS are a class of powerful oxidants, the most reactive of which can react with recalcitrant organic molecules at near diffusion limited rates. ROS production in hydrothermal systems may be comparable to the known photochemical yields of ROS in surface waters. The discovery of this abundant hydrothermal source of ROS demonstrates a mechanism for the alteration of refractory organic matter in the deep ocean.

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Significance

The Fe-catalyzed oxidation of sulfide by dioxygen in hydrothermal plumes is shown to be a source of reactive oxygen species (ROS) to the deep ocean. ROS are a class of powerful oxidants, the most reactive of which can react with recalcitrant organic molecules at near diffusion limited rates. ROS production in hydrothermal systems may be comparable to the known photochemical yields of ROS in surface waters. The discovery of this abundant hydrothermal source of ROS demonstrates a mechanism for the alteration of refractory organic matter in the deep ocean.

The reduction of dioxygen by ferrous complexes is the initiating step for a chain of reactions (Eqs. 1–3) that is among the most important sources of reactive oxygen species (ROS) in the aquatic environment (1). ROS are a class of powerful oxidants that include the hydroperoxyl radical (HO2) and its conjugate base (superoxide, O2−), hydrogen peroxide (H2O2), and hydroxyl radical (HO). Solar-induced photoprocesses with or without Fe(II) or Mn(II) to generate H2O2 (Eqs. 1, 2, and 3). The inventory and production rates for H2O2 in surface seawater are the highest of the ROS (2). However, hydroxyl radical is the most reactive of the ROS and can rapidly oxidize most organic molecules (1, 3). The production of H2O2 can support the subsequent production of hydroxyl radical by the reaction of H2O2 with Fe(II) through Fenton and photo-Fenton reactions (1). This process creates a link between the carbon cycle and the sources of Fe(II) and H2O2 to oxygenated waters in the ocean (1).

The rate of the reaction between Fe(II) and H2O2 (Eq. 3) at typical seawater pH values is competitive with the rates reported for the reaction of Fe(II) with O2 (Eq. 1) (4, 5). In contrast, the rates for photoproduction of hydroxyl radical via the photolysis of precursors are relatively slow (1). Thus, in surface seawater, the rate of photoproduction of Fe(II) is an important factor contributing to the intensity of hydroxyl radical production and the associated abiotic oxidation of organic carbon (1). However, Fe(II) is also produced by microbial processes in the oceans, including the action of facultative anaerobes, and is a critical intermediate in the catalytic oxidation of hydrogen sulfide by dioxygen (6, 7). Microbial processes at redox interfaces have been well established as sources of reductive equivalents that lead to ROS production, including O2− and H2O2 (6–8). While photoprocesses have generally been accepted as the more significant source of H2O2 and Fe(II), hydrothermal systems (vents and seeps) are major, direct sources of reduced Fe and S species to the oceans (9, 10). Given the kinetics of the Fenton process (1, 5), the venting of hydrothermal fluids should support a very high intensity of ROS production and organic carbon mineralization in hydrothermal plumes.

The reduction of Fe(III) by H2S in the plumes and the corresponding abiotic oxidation of Fe(II) species (e.g., Fe2+ (aq), FeS0) should maintain the ROS production cycle until the available reduced sulfur species have been oxidized (Eqs. 1–4). The corresponding yield of ROS would be limited to the summed total of reactive equivalents injected by H2S and Fe(II) species (Eq. 5). This process would be analogous to that seen in coastal pore waters but not limited by the rate of microbial metabolism (6–8, 11). Superoxide, generated via Fe(II) oxidation, could then react with Fe(II) or Mn(II) to generate H2O2 (Eqs. 1, 2, and 6) (8, 12). While Fe(II) in vent water should exist primarily as sulfide phases (e.g., FeS clusters), ambient Fe(III) can react catalytically with reduced S species (e.g., H2S and FeS). These, in turn, can
regenerate Fe(II) and maintain the ROS production chain (Eq. 4), as long as reduced S species are available (12). Similarly, some of the produced superoxide can react with ambient Fe(III) and regenerate Fe(II) (the back reaction in Eq. 1) and still maintain the ROS production cycle. The physiochemical processes leading to these unique reaction mixtures are depicted conceptually in Fig. 1, and the measured reactants and products are presented in Table 1. The maintenance of the Fe cycle by reduced S species can also support a chain reaction, resulting in additional ROS production including hydroxyl radical (1, 6, 7). Hydroxyl radical can readily react with refractory molecules that are kinetically inhibited from direct reaction with O2 (13).

The potential scale of ROS production in hydrothermal plumes is a function of the flux of reduced sulfur species from vents and the bottom-water O2 that mixes within the plume (Eq. 5) to maintain the Fe(II)/Fe(III) cycle (9, 10, 14–17). As noted above, Fe(II) and/or reduced sulfur species are typically the limiting reactants in seawater. The hypothesis tested here is that O2 is the limiting reagent during the initial mixing of hydrothermal vent waters with ambient seawater to form the plume.

$$\text{Fe(II)}_{\text{hot}} + O_2 \leftrightarrow \text{Fe(III)} + O_2^- \quad \text{(oxygen reduction)}$$  \hspace{1cm} [1]

$$\text{Fe(II)}_{\text{hot}} + O_2^- + 2H^+ \rightarrow \text{Fe(III)} + H_2O_2 \quad \text{(superoxide reduction)}$$  \hspace{1cm} [2]

$$\text{Fe(II)} + H_2O_2 + H^+ \rightarrow \text{Fe(III)} + H_2O + HO^- \quad \text{(Fenton reaction)}$$  \hspace{1cm} [3]

$$2\text{Fe(III)} + H_2S \rightarrow 2\text{Fe}^{3+} + S^0 + 2H^+ \quad \text{(sulfide oxidation sustained Fe(II) regeneration)}$$  \hspace{1cm} [4]

$$O_2 + H_2S \rightarrow S^0 + H_2O_2$$  \hspace{1cm} (sulfide induced ROS formation as the sum of Eqs. 1, 2, and 4), [5]

$$\text{Mn(II)}_{\text{hot}} + O_2^- + 2H^+ \rightarrow \text{Mn(III)} + H_2O_2 \quad \text{(Mn(II) oxidation)}$$  \hspace{1cm} [6]

Accepting that the plume inventory of H2S can maintain the Fe cycle (Eq. 4), then sulfide oxidation in the plume would set an upper limit on ROS production arising from the vent flux of reduced Fe and S species (Eq. 5) (6, 7). Eq. 5 shows the expected reaction chain intermediate, H2O2, resulting from the net cycle of metal-mediated H2S oxidation as the sum of reactions depicted in Eqs. 1, 2, and 4. Fig. 2 depicts the intersection of the Fe and S oxidation cycles with the full ROS reaction chain in plumes. The cycles include pathways for ROS-mediated reactions with dissolved organic carbon (DOC) that lead to hydroxylated aromatic and aliphatic organic molecules in vent systems (18). Hydrogen peroxide is a relatively long-lived intermediate in the ROS reaction chain depicted having a half-life of days in seawater where Fe(II) species are limiting (6, 19). Hydrogen peroxide was selected as an ROS production indicator for this study because it is the rate-limiting intermediate between the very short-lived ROS in the reaction chain, superoxide and hydroxyl radical. The proposed ROS production cycle was investigated by measurement of a suite of reactants and products in plume waters using a series of samplers and sensors deployed from the human occupied submersible Alvin, at 9°N East Pacific Rise (EPR).

### Results

**ROS Production in Vent Plumes.** H2O2 was measured on samples from three different Alvin dives (Table 1). One sample reference set was collected in the deep-water column for comparative purposes and two at vent locations, low-temperature Q-vent and high-temperature Biovent at 9°N. The amplex red technique was employed to measure hydrogen peroxide in the samples in situ (20). Syringe samplers (modified from ref. 21) were precharged with amplex red and horseradish peroxidase (HRP) in a phosphate buffer and deployed in the Alvin equipment basket. Samples were drawn through a Teflon sipper tube into the syringes where H2O2 drove the conversion of amplex red to the stable product resorufin, which was visible in syringes immediately upon collection. The concentration of resorufin was later measured fluorometrically upon return to the ship (20).

All samples from vent-affected waters (within plumes or associated diffuse flow fields) showed statistically significant (relative to background) hydrogen peroxide concentrations ranging from 1.1 to 6.2 μM. Reference deep-water samples, collected away from vent-affected waters, did not contain statistically significant amounts of hydrogen peroxide relative to blanks ≤ 200 when premeditated with H2O2. Samples without reagents were also collected from the same position in plumes for determination of ambient Fe and Mn species. Fe(II) and Mn(II) were present in every plume sample collected. Mn(III) was detected in several plume samples but Fe(III) was not. The sipper used for H2O2, Fe, and Mn sample collection was attached to an electrochemical probe that provided measurements of O2 and sulfide phases in situ by cyclic voltammetry (22). In most cases, voltammetric measurements were taken synoptically with the discrete syringe sampling. Temperature limitations on the probe precluded sampling near the high-temperature vent fluid endmembers (e.g., 360 °C at biovent). However, separate discreet samples were collected with Ti samplers in the plumes, allowing the measurement of temperature, bromide, iodide, and additional sulfur species including S0 at most sites and temperatures (23).

The concentration of reduced Fe(II) species in the plume samples correlated positively with temperature consistent with the input of reduced Fe species in the vent fluid (Fig. 3). The concentration of H2O2 correlated inversely with temperature in the plume for both vent locations (Fig. 3), and the regression indicated that H2O2 was not detectable above 39 °C. At high temperature, the direct reactions of H2O2 would be kinetically fast in the presence of excess Fe(II) and sulfide and support a very intense ROS production chain but limit the H2O2 inventory. The absence of detectable H2O2 in ambient reference bottom water (Table 1) precluded seawater mixing as a source for the high-plume H2O2 inventory. The increase in H2O2 as a function
of plume mixing with bottom water was consistent with ROS production during oxidation of reduced Fe species as depicted in Fig. 3. On cooling in the plume, further reactions of $H_2O_2$ were kinetically slower. As the plume aged and temperature decreased, Fe(II), sulfide, and other reductants were lower in concentration and led to a longer residence time of the $H_2O_2$ intermediate which was observed as a larger inventory.

Superoxide production was not measured directly due to matrix interferences in the plume and expected short lifetime (i.e., low concentration) under plume conditions. However, the presence of hydrogen peroxide alone was evidence for the production of superoxide, an essential intermediate for hydrogen peroxide production (12). Superoxide would not be expected to have a long lifetime in the plume environment given its reactivity with Fe(II) or Mn(II) (Eq. 6, similar to Eq. 2).

The one-electron oxidation of Mn(II) to Mn(III) by oxygen is thermodynamically unfavorable (12, 24), but the reaction of Mn(II) with superoxide is favorable and is rapid based on the rate constant for the Mn(II) reduction of superoxide to $H_2O_2$ ($k = 1 \times 10^6 M^{-1} \cdot s^{-1}$ versus $1.5 \times 10^5 M^{-1} \cdot s^{-1}$ for Fe(II)) (25). A number of plume samples taken in conjunction with $H_2O_2$ and led to a longer residence time of the $H_2O_2$ intermediate that was observed as a larger inventory.

$$F_b = 1 - \left(\frac{1 - \left[O_2\right]}{1 - \left[O_2\right]}\right),$$  \[7\]

where $T_p$ was the plume temperature, $T_b$ was the bottom-water temperature, and $T_v$ was the vent fluid temperature. The fraction of bottom water (as percent) and predicted initial oxygen concentration (equivalent to $O_2$ consumed as $O_2$ measured was typically below the detection limit) were plotted against measured $H_2O_2$ for Q-vent (Fig. 4).

**Discussion**

The strong correlation between initial oxygen concentration and $H_2O_2$ (Fig. 4) suggested that $O_2$ was the limiting reactant (Eqs. 1 and 2) during initial mixing in the plumes. The experimentally derived rate constant for the oxidation of Fe(II) by dioxygen is on the order of $10^5 M^{-1} \cdot s^{-1}$ under the condition solutions interrogated in this study (4, 7). The absence of detectable Fe(III) species was consistent with the forward reaction (Eq. 4) and rapid production of superoxide (Eq. 1) following reduction of superoxide by Mn(II) (25) or sulfide (26). Within 0.5 m of the Q-vent orifice ($\sim 25 ^\circ C$), the maximum $O_2$ concentration (and thus $H_2O_2$ that

**Table 1. Dives and sample locations with physical and chemical parameters measured. Abbreviations used in the table are: Plume-xM-vent plume and approximate height above orifice; Free $\Sigma H_2S = [H_2S] + [HS^-].$**

| Dive No. | Location | $T$ °C | $O_2$ μM observed | $O_2$ μM predicted | $H_2O_2$ μM | Fe(II) μM | Mn(II) μM | Mn(III) μM | pH | Free $\Sigma H_2S$ μM |
|---------|----------|--------|--------------------|--------------------|-------------|-----------|-----------|------------|----|--------------------|
| 4888    | Water Column | 1.85   | 105                | 105                | below detection | limit | not measured | not measured | 16.6 | not measured |
| 4888    | Biovent Diffuse Flow | 15.5   | below detection | 100                | 4.5          | not measured | not measured | not measured | not measured | 10.9 |
| 4888    | Biovent Diffuse Flow | 15.5   | below detection | 100                | 2.9          | not measured | not measured | not measured | not measured | 5.79 |
| 4888    | Biovent Plume-1 m | 9.5    | below detection | 102                | 2.9          | not measured | not measured | not measured | not measured | 45.8 |
| 4888    | Q-Vent Plume-3 m | 6.0    | 10                 | 94                 | 5.2          | 49         | 21.4      | 3.93       | not measured | 31.2 |
| 4888    | Q-Vent Plume-<1 m | 9 to 11 | below detection | 82                 | 4.7          | not measured | not measured | not measured | not measured | 32.9 |
| 4888    | Q-Vent Plume-1 m | 2.0    | 80                 | 105                | 6.2          | 21.2      | 9.75      | below detection | limit | not measured |
| 4890    | Biovent, Plume-<0.5 m | 25 to 30 | below detection | 96                 | 2.2          | not measured | not measured | not measured | not measured | 223 |
| 4890    | Biovent, Plume-<0.5 m | 27 to 30 | below detection | 97                 | 2.8          | 289       | 4.75      | 10.2       | 6.60       | 250 |
| 4890    | Biovent, Plume-1 m | 5 to 9  | below detection | 102                | 5.8          | 8.30      | 8.96      | 7.69       | 6.28       | 72.6 |
| 4890    | Q-Vent, Plume-<0.5 m | 20 to 30 | below detection | 39                 | 1.1          | not measured | not measured | not measured | not measured | 186 |
| 4890    | Q-Vent, Plume-<0.5 m | 20 to 30 | below detection | 39                 | 2.3          | 273       | 100.3     | below detection | limit | 5.42       | 81.8 |
| 4890    | Q-Vent, Plume-1 m | 7 to 8  | below detection | 87                 | 6.2          | 134       | 49.8      | 6.14       | 5.81       | below detection limit |
ROS Production Chain Maintained by Fe and S Cycles in the Plume

Expected Plume Products

Bottom Water Mixing

Fe(II) → Fe(III) → Mn(II) → Mn(III) → H2O2 → HO·, ROH, ROO·

Hydrothermal Flux

ΣS_red, Fe(II)tot

Fig. 2. The intersections between the Fe, S, and ROS cycles. The coinjection of reduced Fe and S species from vents into oxic bottom water generates plumes that support extended ROS generation in the deep ocean, enabling cooxidation of other dissolved species. R = ambient molecular species, for example, DOC. Red indicates hydrothermal inputs, blue indicates plume products, and bold indicates measured species. Black indicates bottom-water species.

could be produced) was predicted to be 39 μM based on Eqs. 1 and 2 and the mixing of seawater with plume water (Eq. 7). However, the H2O2 was about 2 μM, consistent with the rapid reaction with ambient Fe(II) (1) and other reductants in the plume [e.g., H2S and S(0)]. As temperature decreased to 2 °C, the measured H2O2 concentration increased as available reductants decreased in concentration (H2O2 production became greater than consumption), and reactions were slowed. The instantaneous concentration of H2O2 measured could easily have been maintained by the cyclic oxidation of measured Fe(II)- or Mn(II)-containing species (Eqs. 2 and 6) in conjunction with ambient sulfur species (Eq. 5) (6, 7, 26). Eq. 5 predicted a similar trend for H2O2 and S(0) production in the plume. The concentration of S(0) was measured at several sites during the cruise (23) but only overlapped with ROS sampling during dive 4890 and only at biovent. The range of S(0) concentrations in the Biovent plume was 1 to 19 μM (23), very close to the range of the measured H2O2 for that dive (2.2 to 5.8 μM, Table 1). While Fe(III) was the likely oxidant for H2S in the plume, the absence of detectable Fe(III) in the recovered plume water was expected where an excess of H2S would sustain the Fe(II) cycles (Eq. 4) (6, 7, 12, 23). These results were consistent with coupling of the Fe and S cycles in support of ROS production as depicted in Fig. 2.

An estimate of the efficiency of H2O2 production during metal-mediated sulfide oxidation in plumes was derived from the slope of the correlation for the initial O2 input and measured H2O2 from Fig. 4. The slope of the correlation yielded a predicted production efficiency of ~6.5% on a mole/mole basis, indicating the intensity of consumption reactions for O2 and/or H2O2 during initial plume mixing. The negative intercept for H2O2 was consistent with the short lifetime of H2O2 under plume conditions that resulted in an underprediction of the efficiency of the ROS chain propagation. However, even at 6.5% production efficiency, the reduction of O2 by reduced vent species [e.g., sulfide and Fe(II)] accounted for a major source of H2O2 to the deep ocean. Subsequent reduction of the resulting Fe(III) by excess sulfide as the plume rose would have continued to produce H2O2 and, at the measured pH range of the plume (Table 1), the more reactive hydroxyl radical (Eq. 3) (1, 5–7, 25–28).

Hydroxyl radical is an unselective oxidant that can be reduced by organic and inorganic species often at nearly diffusion-controlled rates. Two factors may be critical to the impact of hydroxyl radical production in plume waters on DOC lifetimes: the intensity of ROS production and the fraction of that production available for reactions with DOC. The intensity of ROS

H2O2 production at Q vent

Fig. 3. [Fe(III)] and [H2O2] versus temperature of the plume waters for both vent sites where samples were taken. Note the positive correlation for [Fe(III)] and the negative correlation for [H2O2].

Fig. 4. Initial [O2] in the Q-vent plume waters was calculated from mixing of vent water with bottom water using temperature as a conservative parameter. The high correlation indicates production of H2O2 in the plume. The vent fluid endmember (40 °C) was plotted assuming [H2O2] and [O2] = 0 μM.
hydrothermally derived ROS and the initial comparison it affords suggests these nonphotochemical sources of ROS may be comparable in magnitude to surface waters. Given the relative spatial heterogeneity of plume sources, the local intensity of ROS cycling adjacent to hydrothermal vents may be among the highest yet measured in a natural system.

**Implications with Respect to the Global C Cycle.** The oceanic carbon reservoir, as refractory DOC, is on the same order of the carbon reservoir (as CO₂) in the atmosphere, and processes that mediate the mineralization of DOC to CO₂ are critical to the global carbon cycle. While it has been proposed that the oceanic inventory of refractory DOC is controlled by photochemical degradation in surface waters (33), the deep refractory pool of DOC (>1,000 m) is less photochemically reactive than shallower material (34). Photochemically mediated production of ROS leads to near-surface ocean enrichments of hydrogen peroxide in the 20 to 80 nanomolar range (35) and may contribute to the mineralization of DOC fractions via photo-Fenton reactions (1, 3, 34). However, the abundance of these species is low compared to that measured in hydrothermal plumes, and ambient surface ocean pH (~8) is less favorable to production of the more reactive ROS, hydroxyl radical. In contrast, high-hydrogen peroxide concentrations and the lower-pH values observed in plumes are more consistent with much more reactive natural systems like cloud waters (36) and engineered systems (30). The depleted DOC inventory of about 14 μM measured previously in the high-temperature plume at the EPR (18) is consistent with the hydrothermal alteration/decomposition of nonaromatic compounds (37) during hydrothermal circulation. The observed increase in hydroxylated aromatic compounds in plumes from this and other vent locations (18, 38, 39) suggest that this abiogenic oxidation process is not unique to this hydrothermal system and is related to the high intensity of hydroxyl radical production and reactivity reported here. Overall, the measured chemical inventories of ROS in plumes indicate an efficient flow-through reactor for refractory DOC in the oceans.

In summary, hydrothermal plumes represent a source of ROS to the deep ocean. The measured H₂O₂ production, pH values, and abundant reduced S and Fe species in plumes indicated conditions likely to result in significant ROS production, as H₂O₂, under conditions likely to support hydroxyl radical production as well (25, 26). Hydroxyl radical reacts with organic molecules at essentially diffusion-controlled rates (26) making plumes a possible sink for even the most refractory DOC and POC in the deep ocean. Oxidized derivatives of benzoic acid (2,3-dihydroxybenzoic acid, a catechol, and 4-hydroxybenzoic acid) and the organic sulfur compound 2,3-dihydroxypropene-1-sulfonate have been reported in plumes (18) as have increases in O/C ratios and polyphenolic compounds (38), all circumstantial evidence for a role for ROS in the fate of refractory carbon. The hydrothermal ROS production estimated here would have a major impact on the oceans DOC inventory. In this context, it is not surprising that the measured ages of DOC in the deep ocean (40, 41) are on the same order as the estimates for the circulation time of the ocean through vent plumes (14).

**Materials and Methods**

**Plume and Diffuse Flow Sampling.** The vent fields at 9 North on the EPR were accessed with the human-occupied submersible Alvin. Water samples from plumes and a diffuse flow area were collected with 1/8-inch or 3.175-mm Teflon tubing housed in a delrin wand that could be positioned by a manipulator of Alvin. Samples were collected in syringes set in a modified Jahnke syringe sampler (21) that was remotely triggered. The wand above the plumes and a diffuse flow area were collected with 1/8-inch or 3.175-mm Teflon tubing housed in a delrin wand that could be positioned by a manipulator of Alvin. Samples were collected in syringes set in a modified Jahnke syringe sampler (21) that was remotely triggered. The wand above the plumes was set up in pairs with one of the pair for water collection for shipboard analysis of Fe and Mn species and the second of the pair preloaded with reagents for production of a hydrogen peroxide signal.
Hydrogen Peroxide Measurement. Hydrogen peroxide concentrations in plume samples were analyzed by the peroxidase-catalyzed conversion of amplex red to resorufin (21). Reaction solutions were made up daily from preweighed frozen solid for HRP and preweighed salts for buffers. Amplex red solution was made up from premeasured vials containing stock 10-acetyl-3,7-dihydroxyphenoxazine dissolved in DMSO. While the precision of the measurements was better than 5%, procedural blanks varied considerably with variations in shipboard deionized (DI) water quality, typically ranging from 50 to 100 nM. This was probably associated with the ultraviolet (UV) disinfection system used for the water supply. The worst-case data from these measurements was used to estimate a conservative minimum detection limit (MDL) of 200 nM for the shipboard results.

Water samples were drawn into 35-mL syringes that were precharged with 8 mL of a mixture of HRP at an activity of 0.4 U/mL and amplex red at a concentration of 100 μM in 0.05 M Phosphate buffer (pH 7.0) to the reduced Fe and S measured in the samples) made up in prefiltered seawater adjusted to pH 7.4. Thus, Fe(III) binding to phosphate was much faster than the reaction of Fe(II) with H2S, limiting the impact of the possible interferent. Reagent concentrations were previously validated for systems containing high Fe(II) and sulfide loading (50). Cyclic Voltammetry Measurements. A DLK-SUB IV electrochemical analyzer from Analytical Instrument Systems, Inc. was used to collect O2 and H2S data via a cable from Alvin basket at 1.6 °C in order to keep their temperature constant. The wand was positioned by a manipulator from Alvin and suggestions that have improved this manuscript.

Cyclic Voltammetry Measurements. A DLK-SUB IV electrochemical analyzer from Analytical Instrument Systems, Inc. was used to collect O2 and H2S data (22). Briefly, this instrument contained a potentiosat, a multiplexer (to monitor up to four solid-state Au amalgam [AuHg] working electrodes one at a time), and a computer, which communicated with another computer in the Alvin. The electronics were enclosed in a titanium housing and powered via a cable from Alvin. A Delrin wand housed the AuHg-working electrodes, which were cabled to the analyzer, and 1/8-inch Teflon tubing, which allowed sampling via the syringe sampler. The wand was positioned by a manipulator from Alvin; the solid-state Ag/AgCl reference and Pt counter electrodes in the Alvin baseline at 1.6 °C in order to keep their temperature constant. The wand was positioned by a manipulator from Alvin. For each discrete location sampled by syringe, 5 to 7 voltammetric scans were taken and the data averaged. The potential range scanned was −0.1 to −1.8 V to −0.1 V at a scan rate of 2,000 mV s−1. A 5-sec conditioning step at −0.9 V and another at −0.1 V for 2 s was applied before each scan to electrochemically clean the Hg surface. The minimum detection limit (MDL) of 200 nM for the shipboard results.

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