

Documenting the suboxic zone of the Black Sea via high-resolution real-time redox profiling

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Received 1 November 2003; accepted 28 March 2006

Available online 6 September 2006

Abstract

We coupled an in situ electrochemical analyzer to a CTD pump profiler system to measure redox species across the oxic–anoxic interface of the Black Sea water column. Voltammetry was performed using gold–amalgam working electrodes to measure simultaneously oxygen and dissolved sulfur species (S_8 , S_x^{2-} , HS^-/H_2S) both in situ (at < 1 m vertical intervals) and in an on-deck flow cell attached to the outflow of a pump profiler (vertical resolution of about 1.5 m). In situ data agreed with measurements made in the flow cell and with measurements made from samples collected by rosette bottle casts. In situ voltammetry provided undisturbed, high-resolution measurements, and revealed significant yet subtle features not seen by traditional methods because of small spatial separation between the features and the measurements. Layers of oxygen intrusion (< 5 m thick, from 10 to 150 μM O_2) were present within the suboxic zone of the southwest Black Sea that are not present in the west-central and northeast Black Sea. Oxygen injection also occurs at other depths throughout the southwest and corresponds with small temperature anomalies, suggesting influence by Bosphorus inflow up to 150 km from its entrance to the Black Sea. Such an inflow of oxygen, as well as spatial variations of the halocline, affect both manganese and, subsequently, sulfide oxidation for a large portion of Black Sea intermediate water (H_2S onset occurred ~60 m deeper in the southwest as compared to the west-central). In situ voltammetric analyses provided rapid redox information, thus enabling more accurate targeting of specific geochemical features by the CTD rosette package.

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Keywords: In situ voltammetry; Oxygen; Sulfide; Suboxic; Black Sea; Redox

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1. Introduction

Oxygen and sulfide were commonly thought to coexist in the transition from oxic to sulfidic waters in the Black Sea (Sorokin, 1983; Fiaschuck and Ayzatulkin, 1986), until 1988 when a suboxic zone

with nondetectable levels of O_2 and H_2S (Murray et al., 1989) was observed. More recent studies have confirmed vertical separation between oxygen and sulfide in the Black Sea water column (Tugrul et al., 1992). Natural barriers inhibiting vertical fluxes are established through density stratification of the water column (Sorokin, 1983; Anderson et al., 1988; Murray et al., 1995; Pers and Rahm, 2000; Scranton et al., 2001), caused by sharp increases in salinity (Anderson et al., 1988) and/or decreases in temperature (Scranton et al., 2001). In the case of the Black Sea, a vertical transition between oxic and anoxic waters is likely to have existed for over 7000 years (Arthur and Dean, 1998; Lane-Serff et al., 1997), since post-glacial saline Mediterranean waters broke through a land barrier and flooded the isolated freshwater Black Sea basin at a rate of 0.1–1 m per day, setting up a permanent pycnocline. The nature of the physical stratification and the subsequent steep chemical gradients make accurate sampling of these constituents around the redox transition problematic, yet crucial to understanding the overall redox budget of the Black Sea.

Traditional methods for water sampling use casts with bottles mounted on a CTD rosette. Such rosette casts induce water mixing during the upward and downward motion through the water column. Furthermore, water samples are collected in vertically oriented bottles, which integrate waters over a vertical distance of greater than a meter. In 1988, a pump profiling system was deployed in the Black Sea (Codispoti et al., 1991) to provide continuous vertical profiling capability, but, again, mixing may be induced through pumping of water from depth to the ship's lab through 200 m of nylon tubing. Reactions may occur as a result of particulate adhesion to the tubing walls. Modern deployable voltammetric analyzers coupled to Au/Hg electrodes recently have been used to characterize O_2 , H_2O_2 , H_2S , S_x^{2-} , soluble S_8 , Fe(II), Fe(III), aqueous FeS, Mn(II) in salt marsh (Bull and Taillefert, 2001), continental shelf sediments and coastal bays (Luther et al., 1999), microbial mats (Glazer et al., 2002), and hydrothermal vents (Luther et al., 2001a,b). Such sensors are capable of making real-time, in situ measurements, independent of sample manipulation, thus providing unprecedented vertical resolution for redox profiling.

Our objective was to apply in situ voltammetric techniques to measure O_2 and H_2S (detection limits of $3 \mu M$ and $30 nM$, respectively), as well as other partially oxidized sulfur species (Brendel and

Luther, 1995; Luther et al., 2001a,b), and provide high-resolution vertical profiling of the oxic–suboxic–sulfidic transition in the Black Sea water column. In addition, an electrochemical flow cell (Luther et al., 2002) was connected to the pump profiler outflow to allow for comparison measurements between in situ and on-deck samples. Data from both of these were compared with data obtained through traditional Winkler (for O_2) and iodometric titrations (for H_2S) of samples taken from separate bottle casts.

We confirm the existence and define the extent and variability of the suboxic zone throughout the western Black Sea. We also demonstrate the existence of partially oxidized sulfur intermediates at depths near the onset of H_2S , and report evidence for lateral intrusions of oxygen-enriched waters into suboxic and anoxic layers using data from the Black Sea cruise on the R.V. *Knorr* (23 May–9 June 2001). We also compare these results with selected data from the 1988 R.V. *Knorr* cruise where the pump profiler system was employed with shipboard hanging mercury drop voltammetry measurements that had a minimum detection limit of $0.1 nM$ for sulfide, as reported by Luther et al. (1991).

2. Methods

Stations within the western Black Sea were occupied during R.V. *Knorr* Voyage 162-legs 16 and 17, between 23 May and 11 June 2001 (Fig. 1). Data were collected to provide new insight and comparisons to the previous major US Black Sea expedition, conducted in 1988 (R.V. *Knorr* Voyage 134, April–July 1988; Murray et al., 1989; Fig. 2).

2.1. Profiling instrument package

The deployment package consisted of two separate instruments attached to a protective stainless steel cage. Temperature, salinity, density, and on-deck flow-through water samples were provided by the pump profiler-CTD system developed by the Monterey Bay and Aquarium Institute (MBARI) (Codispoti et al., 1991). In situ voltammetric analyses were conducted using an electrochemical analyzer built into a pressure housing (Analytical Instrument Systems, Inc.) powered by an internal rechargeable 12 V DC battery. The internal computer communicated with another aboard the ship via a 300-m RS 232 cable and was controlled by an operator or programmed to continuously perform

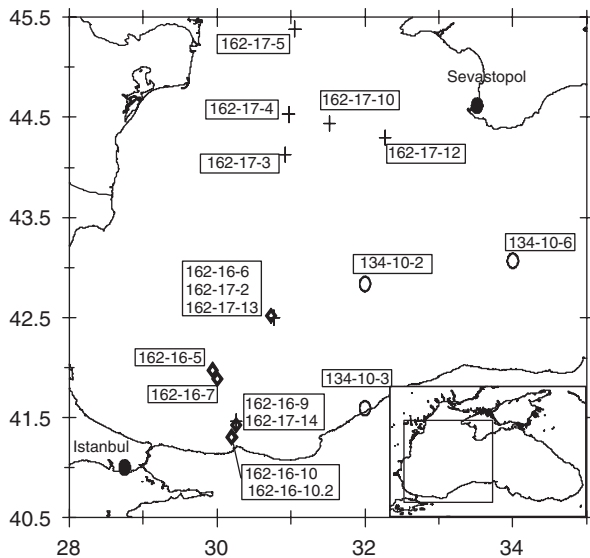


Fig. 1. Selected station locations for the 2001 and 1988 R.V. *Knorr* Black Sea cruises (Voyage 134-10, June 1988, marked by circles; Voyage 162-16, 23–31 May 2001, marked by diamonds; Voyage 162-17, 1–9 June 2001, marked by crosses). Lower right inset locates the area of investigation.

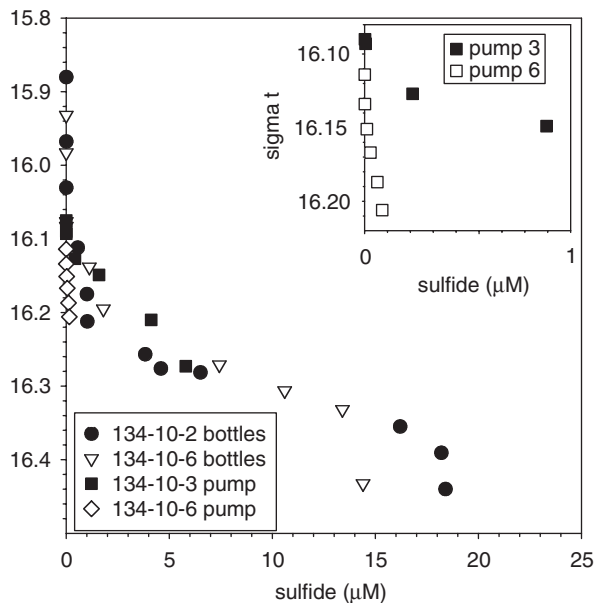


Fig. 2. Interface data for H_2S from stations occupied by R.V. *Knorr* in 1988. The inset magnifies pump profiler data from the interface.

voltammetric measurements. A separate 1-m cable was used to make connections between the working, reference, and counter electrodes and the pressure housing. This cable accommodated up to four

different working electrodes that were used via an internal multiplexer, the counter electrode, and two inputs for the reference electrode so that it could be grounded to ensure signal integrity.

2.2. *In situ* electrochemical analyses

The working electrodes were solid-state gold–amalgam (Au/Hg) and were prepared in polyethylenetherketone (PEEKTM) tubing, sealed with epoxy (Luther et al., 1999). The reference electrode was also solid-state, prepared by oxidizing a 500- μm silver wire in 3 M KCl to make an AgCl coating. Potentials for chemical species measured *in situ* and onboard ship with the same reference electrode were comparable so no pressure effects were observed. Scan rates were 1000 mV s^{-1} . Prior to each scan, conditioning was performed at -0.9 V for 5 s, where none of the chemical species are electroactive (Brendel and Luther, 1995), and then a 2-s deposition at -0.1 V . Potential scan direction was from positive to negative for linear sweep voltammetry (LSV). Cyclic voltammetry (CV) scans were from positive to negative and then back to positive. These conditions provided a detection limit of 30 nM for H_2S and about $3 \mu\text{M}$ for O_2 , as determined by linear regression of known standards. Such low detection levels allowed for the *in situ* identification of irregular multi-layer vertical structure for chemical species, which cannot be detected by traditional methods. Because voltammetric scans could be performed every 9 s or less, measurements were made every 0.8 meter or less based on the descent rate of the MBARI-AIS package through the water column.

At the working electrode surface, O_2 is electrochemically reduced to H_2O_2 at a half-wave potential $E_{1/2} = -0.3 \text{ V}$, and the H_2O_2 is subsequently reduced at $E_{1/2} = -1.3 \text{ V}$, as seen in oxygenated waters above 130 m in the southwest (Fig. 3A–C). The magnitude of the current for the voltammetric wave is proportional to the concentration of oxygen present.

2.3. Discrete sampling and analyses

Aboard ship, a DLK-60 electrochemical analyzer (AIS, Inc.) controlled similar solid-state Au/Hg working, Ag/AgCl reference, and Pt counter electrodes within a flow cell (Luther et al., 2002) attached to outflow from the pump profiling system. Typical conditions were a potential scan range from

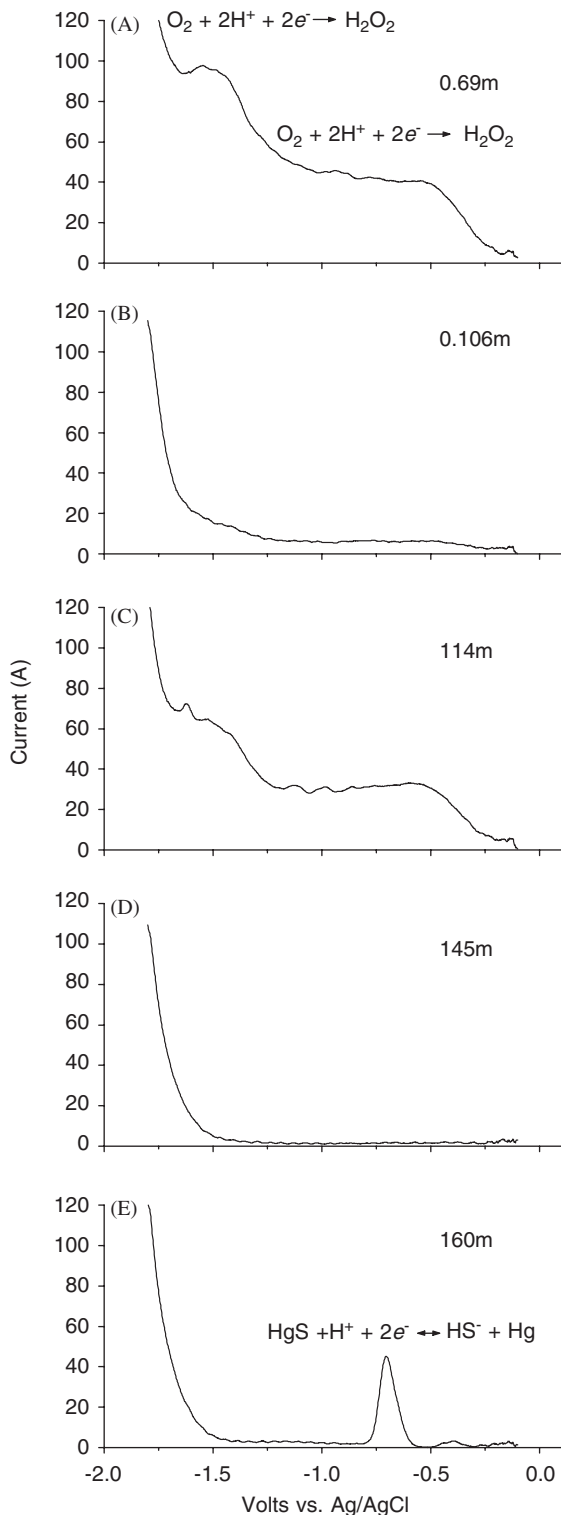


Fig. 3. Representative voltammograms acquired with the in situ electrochemical analyzer depicting vertical structure within the suboxic zone due to inflowing Bosphorus waters at Station 162-16-9, on 29 May 2001.

–0.1 to –1.8 V using LSV or CV modes at 1 or 4 V s^{-1} . The working electrode was conditioned at –0.9 V for 2 s to clean the Au/Hg surface, and then a deposition step at –0.1 V for 5–20 s was performed. These conditions provided the low detection limit of 3 nM for H_2S and about $3 \mu\text{M}$ for O_2 .

Bottle casts were taken immediately before and/or after the in situ profiles. Water samples from discrete bottles were analyzed by Winkler titration for O_2 (detection limit $\sim 3 \mu\text{M}$) and by iodometric titration (detection limit $\sim 3 \mu\text{M}$) and voltammetry (detection limit $\sim 3 \text{ nM}$) for H_2S . Measurements were made for particulate ($> 0.22 \mu\text{m}$) and dissolved Mn from samples collected from Niskin bottles and filtered according to Tebo (1991). Mn in each fraction was determined using the formaldoxine method (Brewer and Spencer, 1971). Particulate elemental sulfur (S_8) was analyzed by ion chromatography after gravity filtering 200-ml samples from a Niskin bottle through a $0.2\text{-}\mu\text{m}$ polycarbonate filter. Filters were kept frozen (-20°C) until they were extracted in 5-mM sulfite solution for 2.5 h to react all elemental sulfur with sulfite to thiosulfate (Luther et al., 1991). The method of (Rozaan and Luther, 2001) was adapted for thiosulfate analysis using anion chromatography with subsequent spectroscopic detection at a wavelength of 215 nm. The eluent was 8 mM perchlorate, at a flow rate of 2 ml/min. Thiosulfate retention time was 7.3 min.

3. Results and discussion

During 23 May–9 June 2001, the R.V. *Knorr* occupied 24 stations chosen to investigate oxidation dynamics across a region influenced by the inflowing Bosphorus Plume. Data from 15 stations are presented here and compared to three stations from the 1988 cruise (Fig. 1). The complete list of participants, stations and data for R.V. *Knorr* 2001 is given at www.ocean.washington.edu/cruises/Knorr2001. Water entering the Black Sea through the Bosphorus Strait (32 m water depth at sill) is more saline and warmer ($12.5\text{--}15^\circ\text{C}$) than the water it entrains from the Black Sea cold intermediate layer (CIL) ($< 8^\circ\text{C}$). These mixed waters create a plume that is injected into layers of the Black Sea below the CIL (Murray et al., 1991), and have been traced to depths of 500–700 m via temperature anomalies (Oguz and Rozman, 1991). This influx of salt is the mechanism for maintaining the main pycnocline of the Black Sea.

3.1. Oxygen and sulfide distributions

Sulfide data are plotted versus density (σ_t) for selected stations from the 1988 R.V. *Knorr* cruise (Fig. 2). At all stations the onset of H_2S occurred near $\sigma_t = 16.1$ (Murray et al., 1995). However, a near-shore location (station 134-10-3) showed a 10-fold or higher sulfide concentration than those observed at the two central locations (stations 134-10-2 and 134-10-6). The samples collected by bottle casts in 1988 had a 5-m vertical resolution and the pump-profiler samples had a 2-m vertical resolution. Interestingly, the sulfide data taken from bottle casts were higher than those from the pump profiler. This may be due to the 1-m length of the bottles that result in water samples with a poorer vertical resolution than the pump profiler, which has a 6-mm diameter orifice at its pump intake.

Based on data in Fig. 2, we decided to design and build an in situ chemical analyzer to determine dissolved O_2 and H_2S and other redox components (Table 1). Deployment of this instrument in 2001 provided in situ voltammetric profiles that helped to document lateral O_2 injection from the Bosphorus Plume (Konovalov et al., 2003). This lateral injection suppresses and distorts the suboxic zone and the upper part of the H_2S onset at stations influenced by Bosphorus inflow throughout the southwest Black Sea, as observed in oxygenated

waters above 130 m in the southwest (Fig. 3A–C). Oxygen concentration is 6 times as high (roughly 40 nA of current, or 150 μM) at 69 m, than at 106 m (roughly 6 nA of current, or 20 μM). An increase in signal current (roughly 40 nA, or 150 μM) signifies an increase in oxygen concentration at 114 m (Fig. 3C), and there is no detectable oxygen signal at 145 m (Fig. 3D). By 160 m, H_2S ($E_{1/2} = -0.6$ V; Fig. 3E, Table 1) is detected.

Oxygen and sulfide profiles for the southwestern station (Fig. 4), from which the voltammograms in Fig. 3 were taken, reveal the degree of vertical structure for the lateral injections. The profiles are plotted against depth to emphasize the narrowness of the lateral intrusions and to match the voltammograms shown in Fig. 3. Intrusions were identified in several individual profiles as narrow local anomalies, three of which can be clearly seen in Fig. 4A and B (84.8–99.6, 110–121, 121–129 m). Fig. 4B is an enlarged view of the full profile in Fig. 4A, and more clearly shows the lateral injections. Oxygen concentrations reflect the same oscillating pattern seen in the voltammetric scans from Fig. 3. In addition, a sharp decrease in sulfide concentration, accompanied by a small temperature increase (162–178 m) following the H_2S onset (151 m, or σ_t of 16.13) suggests that a deeper oxidation intrusion occurred. Such variable, dynamic lateral injections were likely the reason that a well-defined

Table 1

Measurable redox reactions occurring at the 0.1 mm Au/Hg electrode surface vs. the 0.5 mm Ag/AgCl reference electrode

		E_p ($E_{1/2}$) (V)	MDL(μM)
1a	$O_2 + 2H^+ + 2e^- \rightarrow H_2O_2$	-0.30	5
1b	$H_2O_2 + 2H^+ + 2e^- \rightarrow H_2O$	-1.2	5
2a	$HS^- + Hg \rightarrow HgS + H^+ + 2e^-$	Adsorption onto Hg < -0.60	
2b	$HgS + H^+ + 2e^- \rightarrow HS^- + Hg$	~-0.60	<0.1
3a	$S^0 + Hg \rightarrow HgS$	Adsorption onto Hg < -0.60	
3b	$HgS + H^+ + 2e^- \leftrightarrow HS^- + Hg$	~-0.60	<0.1
4a	$Hg + S_x^{2-} \leftrightarrow HgS_x + 2e^-$	Adsorption onto Hg < -0.60	
4b	$HgS_x + 2e^- \leftrightarrow Hg + S_x^{2-}$	~-0.60	<0.1
4c	$S_x^{2-} + xH^+ + (2x-2)e^- \leftrightarrow xHS^-$	~-0.60	<0.1
5	$Mn^{2+} + Hg + 2e^- \leftrightarrow Mn(Hg)$	-1.55	5

Oxygen data were collected by linear sweep voltammetry. Cyclic voltammetry was employed in the absence of oxygen to better measure sulfur species. Potentials can vary slightly with scan rate and concentration. When applying potential from a positive to negative scan direction, sulfide and S(0) react in a two step process: adsorption onto the Hg surface to form HgS, and reduction of the HgS film. Polysulfides react in a three step process: adsorption onto the Hg surface to form a HgS_x film, reduction of the HgS_x film, and reduction of the S(0) in the polysulfide. Increasing scan rate separates electrode reactions 4b and 4c into two peaks because reaction 4c is an irreversible process. Increasing scan rate shifts this signal significantly. MDL = minimum detection limit.

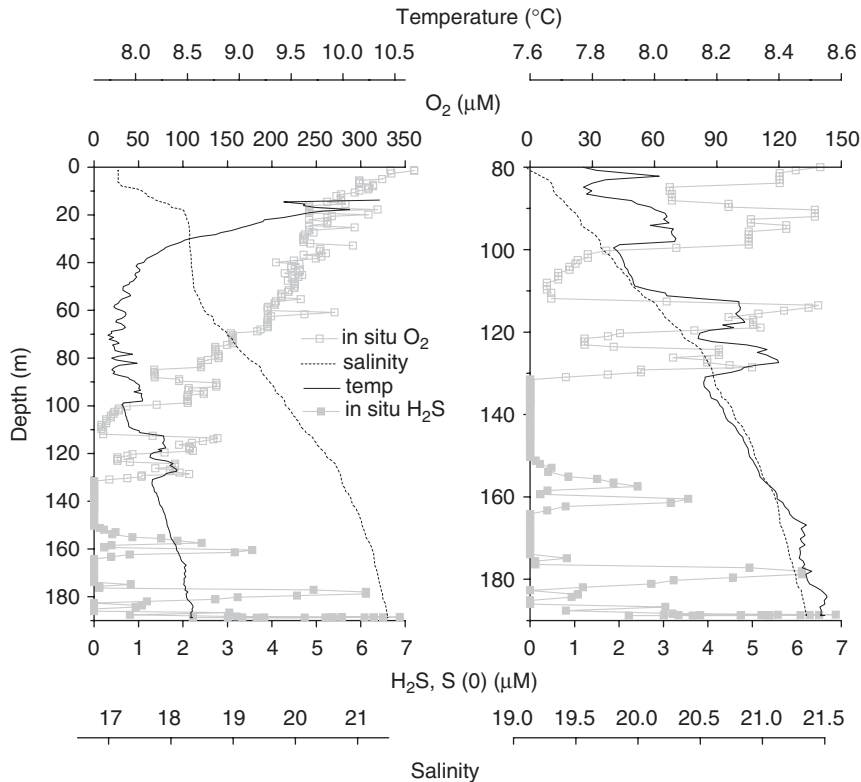


Fig. 4. In situ voltammetry profile of Station 162-16-9, 29 May 2001. Depth versus high-resolution O_2 and H_2S data coupled to temperature and salinity data from the CTD on the MBARI pump profiler.

suboxic zone is not present in the southwest, but is rather undeveloped and less than 15 m thick (~130–145 m).

Our in situ measurements, showed no evidence to suggest that O_2 and H_2S co-exist (e.g., Sorokin, 1972), as none of over 3000 in situ voltammetric scans showed their simultaneous detection in any of the profiles. Furthermore, these data suggest that O_2 penetrates the suboxic zone via lateral mixing from Bosphorus inflow and may be suppressing the onset of sulfide to 150–160 m (σ_t of 16.13–16.23).

The main pycnocline of the Black Sea is present at water densities of $\sigma_t = 14.2$ –16.5 and the core of the CIL is at $\sigma_t = 14.5$. The Bosphorus Plume water is a mixture of 1 volume of the Mediterranean Sea water and 3–10 volumes of Black Sea water (Murray et al., 1991; Ivanov and Samodurov, 2001) so these ventilation layers occur below the CIL ($\sigma_t > 14.5$). Calculations predict the concentration of dissolved O_2 within the Bosphorus plume should reach 100–150 μM (Konovalov and Murray, 2002), which is the approximate concentration in the observed intrusions. Furthermore, these spikes in O_2 concen-

tration coincide with small salinity and temperature increases (< 0.4 °C), also visible in Fig. 4. The anomalies are more readily seen in temperature, rather than salinity, because the relative differences are larger. Linear regressions between temperature and oxygen for each of the three prominent oxygen injection layers reveal significant positive correlations (Fig. 5A–C) and confirm that the warm, salty Bosphorus plume water is the source of the large-scale lateral O_2 flux into the suboxic and anoxic layers of the pycnocline. These linear correlations suggest that the regime of turbulent diffusion, as opposed to double diffusion, is realized for lateral intrusions of the Bosphorus plume. This would hold true for other components as well, i.e. salinity, and is important for understanding dissipation processes of lateral intrusions into the Black Sea ambient waters. It has been argued that physical parameters are adequate tracers of Bosphorus inflow (Latif et al., 1991; Schuf and De Baar, 1995), but because the differences in salinity and temperature between the Bosphorus plume and the ambient Black Sea water are so small and oxygen concentrations differ by

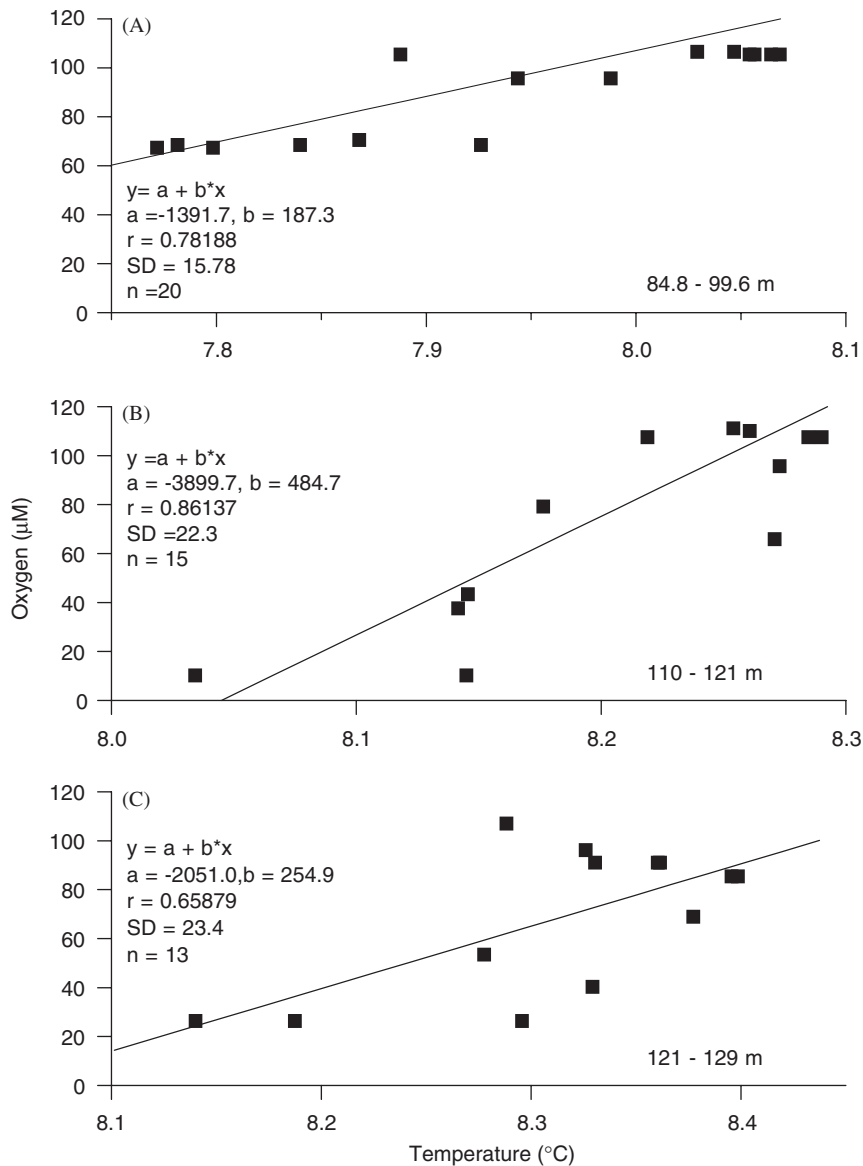


Fig. 5. Linear regressions for temperature and O_2 each of three prominent oxygen injection layers observed at Station 162-16-9, 29 May 2001.

more than $100 \mu\text{M}$, high-resolution oxygen profiling should be the best way to trace lateral intrusions.

In contrast to the frequent, strong disruption of the suboxic layer in the southwest stations, the west-central and northwest stations revealed more stable transitions between oxic and sulfidic waters. And, as a result of the physical gyre circulation (doming of the waters in the central regions; Kononov et al., 2003), the onset of H_2S was considerably shallower at the central station (Fig. 1: 162-16-6, 162-17-2 and 162-17-13) than in the southwest or northwest

stations. At the west-central station (162-17-2), oxygen was nondetectable by 79 m (σ_t of 15.55) and the H_2S onset was at 105 m (σ_t of 16.07) (Fig. 6A). Furthermore, no well-structured lateral oxygen intrusions were visible within the profile, and a stable, well-defined suboxic zone existed for greater than 30 m.

Data from the northwest station (Fig. 1: 162-17-3) illustrates the gyre effect. Oxygen was detected to 101 m (σ_t of 15.45) and the onset of H_2S was not until 139 m (σ_t of 16.10) (Fig. 6B). Note the

consistent values of σ_t between these two stations. Again, no lateral oxygen intrusions are present throughout the profile, and a stable, well-defined suboxic zone exists for nearly 40 m. There is no general shelf-break effect. The anomalies seen at Station 162-16-9 appear to be generated by the Bosphorus Plume alone.

The data in Fig. 6A and B also illustrate the good agreement between in situ voltammetric measurements, on-deck voltammetric measurements in line with the pump profiler outflow, and volumetric titration measurements made from CTD rosette sampling immediately following in situ/pump casts. Minor offsets between flow cell voltammetry and in situ voltammetry may be attributable to time variations in matching waters delivered to the deck from specific density surfaces (depths).

3.2. Reduced sulfur species

Sulfide oxidation results in formation of polysulfides (S_x^{2-}), elemental sulfur (S_8), thiosulfate ($S_2O_3^{2-}$), and polythionates (e.g., tetrathionate,

$S_4O_6^{2-}$), all of which have been recently observed in field studies (Luther et al., 2001a,b). When analyzed with voltammetry, these sulfur species, except for polysulfides) give a single peak. At slow scan rates, H_2S , S_8 , and polysulfides overlap to give a single peak at about -0.6 V (Luther et al., 1985; Wang and Tessier, 1998; Rozan et al., 2000). However, polysulfides are unique because the sulfur exists in two oxidation states and it is possible to discriminate each oxidation state with fast potential scans (Rozan et al., 2000). At a potential more positive than -0.6 V, S_x^{2-} reacts to form an HgS_x species at the Au/Hg electrode, which is an electrochemical oxidation of the Hg. Scanning negatively then results in HgS_x reduction to Hg and S_x^{2-} and this signal overlaps with H_2S and S_8 . The $(x-1)S^0$ atoms of S_x^{2-} are then reduced to sulfide at a more negative potential, depending on scan rate. Since the reduction of S^0 atoms in S_x^{2-} is irreversible, increasing scan rate results in a shift of the peak to a more negative potential, permitting a visual separation of the HgS_x reduction from the $S(0)$ reduction in S_x^{2-} (Table 1).

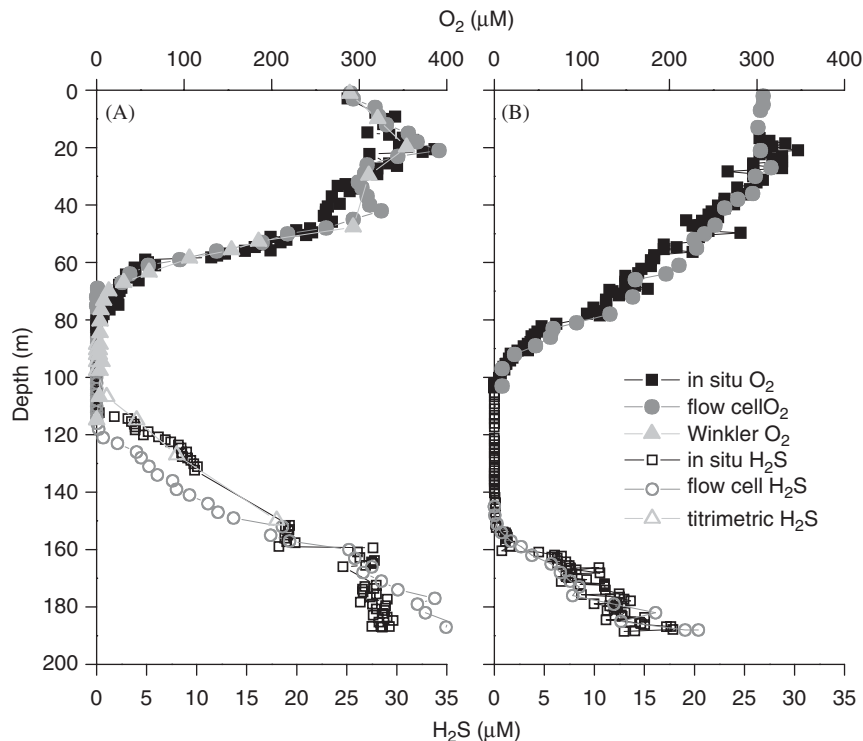


Fig. 6. A. Profile of Station 162-17-2 in the center of the western gyre on 2 June 2001; B. Profile of Station 162-17-3 near the NW shelf break on 4 June 2001. Note differences in O_2 depletion and H_2S onset depths, as well as good agreement between various measurement techniques.

At the base of the suboxic zone at 162–173 (Fig. 1,6B,7) we observed an indication of S_x^{2-} . The voltammetric scan revealed a small, yet discernable split peak at 138.7 m (Fig. 7A and B). In panel A, a clear increase in signal current for HS^- was observed with increase in depth, indicating an increase in sulfide concentration up to approximately $6\mu\text{M}$ at 163 m. The peak shift to lower voltages with higher concentrations is in accordance with the Nernst equation (Luther and Ferdelman, 1993). Closer investigation of the scan at 138.7 m (Fig. 7B) reveals a subtle split peak with potentials of -0.636 and -0.783 V, indicating that $S(0)$ and $S(-II)$ as polysulfide, as well as H_2S were present. Less than 1 m deeper the split peak for S_x^{2-} was not present, indicating that the H_2S boundary had been crossed after descending through a thin layer of S_x^{2-} . The factors that determine the exact depth of this

thin layer are still uncertain but may be related to Mn cycling.

3.3. Manganese distributions

Such partially oxidized sulfur species probably exist throughout the Black Sea at redox transitions where both an oxidant such as $Mn(III/IV)$ and a reductant such as H_2S can be found or where microbial mediation can occur. However, production of sulfur intermediates in Black Sea waters under typical anoxic conditions should lead to their rapid uptake or disproportionation by organisms requiring them for an energy source (Jorgensen et al., 1991). Their transient nature makes the stable suboxic zone of the northwest more ideal for observing them. In contrast, the southwest station exhibited more temporal variability, and therefore intermediate species would be expected to be even more transient and difficult to measure. Four profiles from the southwest stations are shown for 29 May (Fig. 8A), 30 May (Fig. 8B), 31 May (Fig. 8C), and 9 June (Fig. 8D). Profiles are shown versus density to remove doming effects or vertical shifts in the water column and to better resolve variability within the transition between oxic and sulfidic waters (for reference, Fig. 8A shows the same data plotted versus depth in Fig. 6A). Over the 3-day period between 29 and 31 May (Fig. 8A–C), much variability in temperature and oxygen was visible throughout the lower portion of the oxic zone, resulting in a very unstable, undeveloped suboxic zone of less than 5 m thickness. Oxygen disappeared over a range of σ_t from 15.8 to 16.18 (130–155 m). When this location was revisited on June 9 (Fig. 8D), far fewer lateral intrusions were present, resulting in lower overall oxygen concentrations. No well-developed suboxic zone had developed, and while oxygen disappearance was still located at σ_t of 15.99, its depth had shoaled to 122 m. Similarly, the onset of H_2S varied between σ_t of 16.03 and 16.25 (or 120 and 164 m). We note that the shallow H_2S onset at σ_t of 16.03 (125 m, Fig. 8D) was accompanied by only trace levels of H_2S ($<1\mu\text{M}$) and alternating, suboxic layers down to σ_t of 16.33 (152 m), perhaps indicating that an oxidation due to lateral injection had occurred, creating two sulfide zones, similar to those clearly seen in Figs. 8A,3A and 3B.

In contrast, the west-central station showed much less variability in a sequence of different profiles

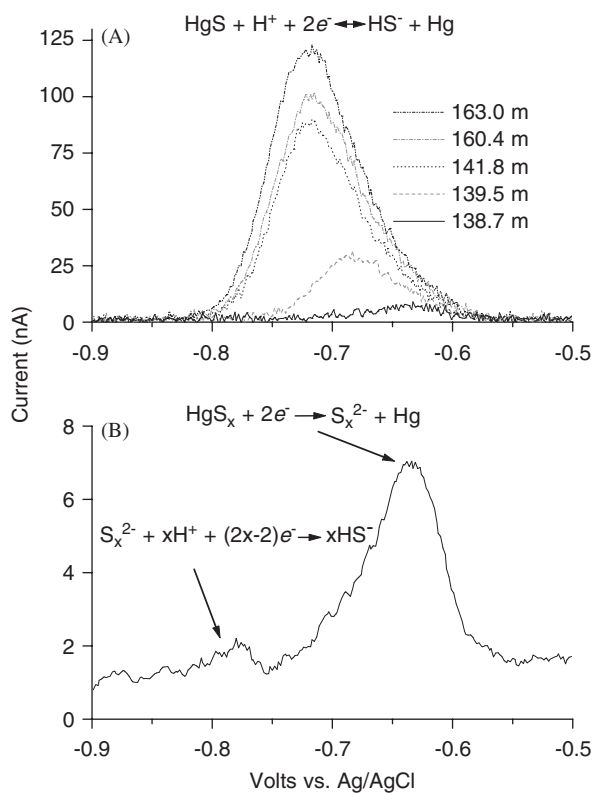


Fig. 7. Representative in situ voltammograms (expanded around HS^- peak) depicting sulfide onset and polysulfide presence at Station 162–173 on 4 June 2001. The peaks observed in panel B consist of a mix of sulfide and polysulfide. The peak at -0.64 V represents both sulfide and polysulfide, while the smaller peak at -0.78 V is attributable to only polysulfide.

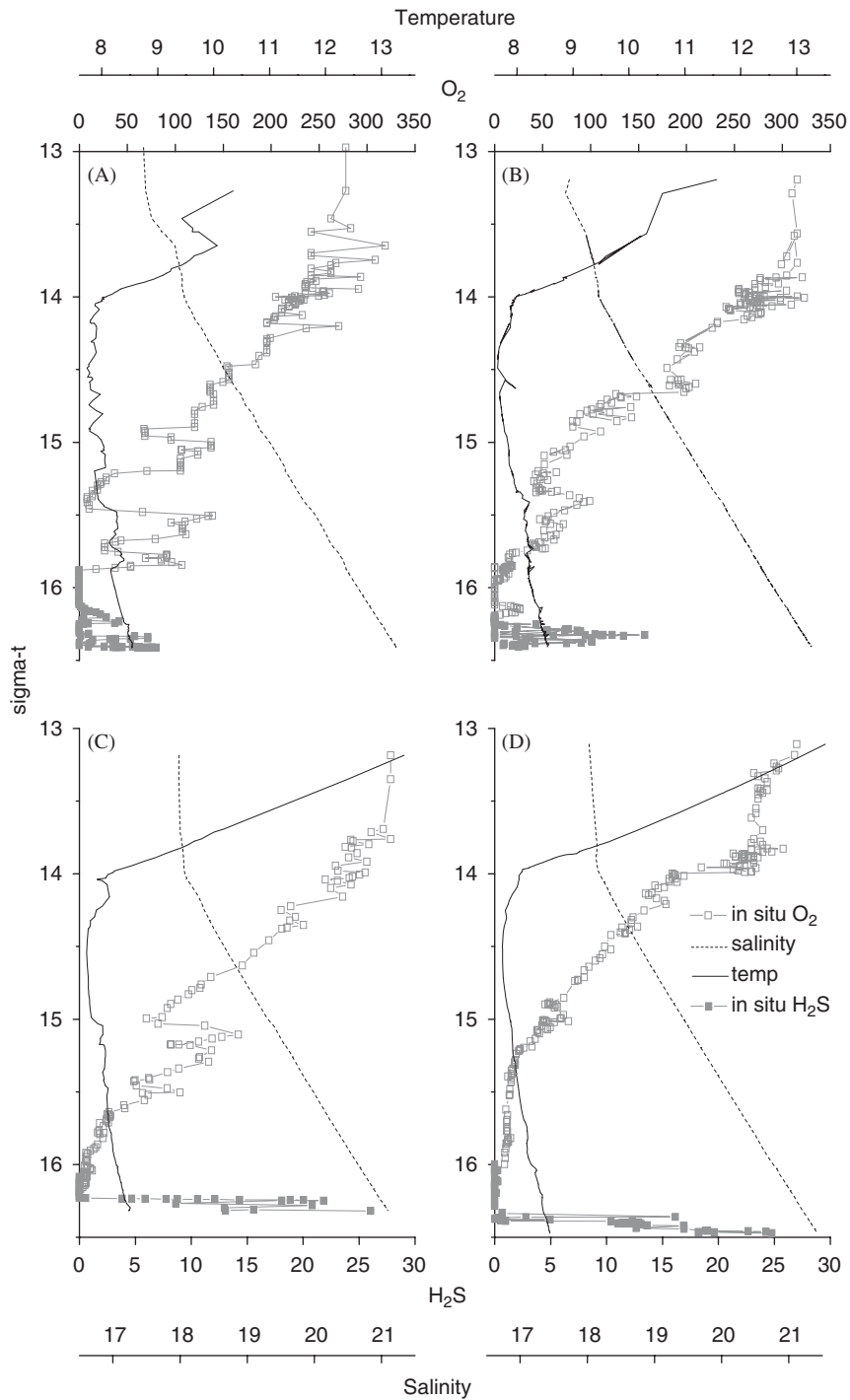


Fig. 8. Density versus high-resolution in situ voltammetric O_2 and H_2S data coupled to CTD data from the MBARI unit in the southwest through time. A. Station 162-16-9 on 29 May 2001; B. Station 162-16-10 on 30 May 2001; C. Station 162-16-10.2 on 31 May 2001; D. Station 162-17-14 on 9 June 2001.

(i.e. no well-established fingers of oxygen intrusion) but did show variability in the density of oxygen penetration versus time. Fig. 9A and B show profiles

from 2 and 8 June, respectively, during which oxygen penetration shoaled from σ_t of 15.55 (79 m) to 15.39 (72 m). H_2S onset stayed relatively constant

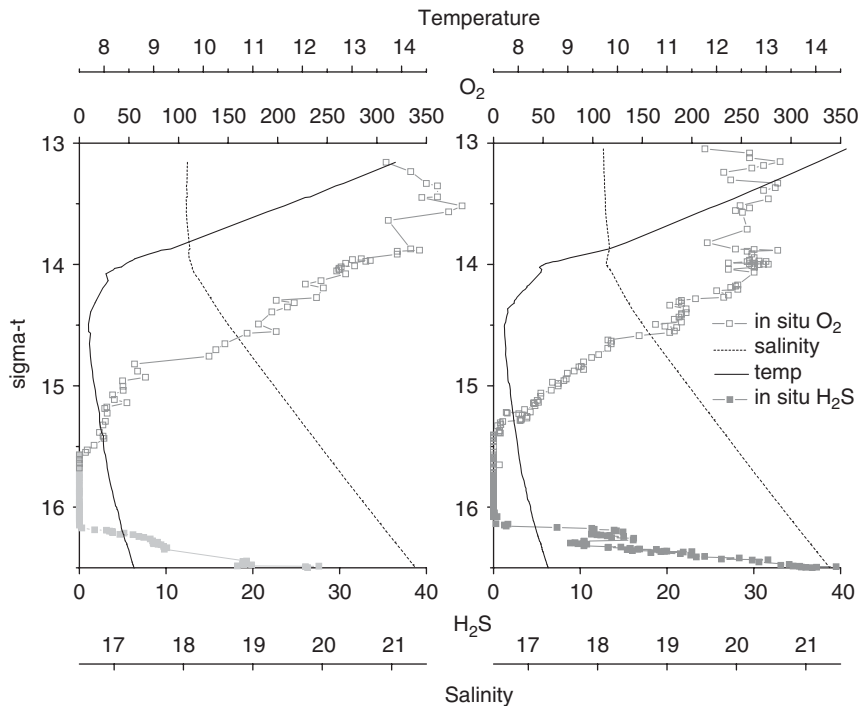


Fig. 9. Density versus high-resolution in situ voltammetric O_2 and H_2S data coupled to CTD data from the MBARI unit for: A. Station 162-17-2 on 2 June 2001; B. Station 162-17-13 on 8 June 2001.

at σ_t of 16.11 (106 m) and 16.07 (105 m). The possible explanation for the depressed oxygen penetration seen on 2 June is lateral oxygen influx, as a small temperature anomaly was observed at σ_t 15.43. Thus, we conclude that lateral influx of oxygen associated with the Bosphorus plume significantly affects the redox budget in the west-central basin, as first proposed by Konovalov et al. (2001) and changes the distribution of the main redox species.

3.4. Suboxic zone distributions and maintenance

Eight in situ voltammetric profiles were made during the 2001 R.V. *Knorr* cruise. In all profiles a suboxic zone was present, but the vertical distributions as well as overall thickness of the suboxic zone varied as a result of proximity to the Bosphorus inflow (Table 2). Comparisons between the west-central, northwest and southwest stations reveal that the depth and density surface at which oxygen disappears and sulfide appears are significantly different (Table 3). The overall thickness of the suboxic zone was significantly less in the southwest

than in the west-central in terms of density, and the p -value statistic of 0.512 were inconclusive for depth. Northwest stations did not vary significantly from west-central stations in any of the pairwise comparisons. Interestingly, northwest and southwest stations only significantly varied in terms of density surfaces for O_2 disappearance and H_2S onset. This is most likely a result of the effect of the Rim Current acting to deepen density surfaces in both the southwest and the northwest (Oguz et al., 1993; Ginzburg et al., 2002).

In situ voltammetry cannot measure particulate species, and its detection limit for dissolved manganese is $5 \mu M$. Thus, traditional wet chemical techniques were employed to investigate the distribution and concentration of dissolved and particulate Mn and particulate S_8 . Dissolved Mn(II) data agree with Lewis and Landing (1991) and Tebo (1991) in terms of onset depth/ σ_t , concentration, and profile shape (Fig. 10B, D, F). In the northwest and west-central Black Sea, the major onset of dissolved Mn was roughly $\sigma_t = 15.8$ – 15.9 , but showed variability and suppression down to $\sigma_t = 16.3$ in the southwest, as seen for O_2 .

Table 2
Suboxic zone thickness in terms of density (σ_t) and depth (m)

Station	Date	O ₂ disappearance		H ₂ S onset		Suboxic zone thickness	
		σ_t	Depth	σ_t	Depth	σ_t	Depth
162-17-3	4 June 2001	15.45	101	16.1	139	0.65	38
162-17-12	7 June 201	15.64	124	16.04	158	0.4	34
162-17-2	2 June 2001	15.55	79	16.11	106	0.56	27
162-17-13	8 June 2001	15.39	72	16.07	105	0.68	33
<i>162-16-9</i>	<i>29 May 2001</i>	<i>15.8</i>	<i>130</i>	<i>16.13</i>	<i>151</i>	<i>0.33</i>	<i>21</i>
<i>162-16-10</i>	<i>30 May 2001</i>	<i>16.18</i>	<i>153</i>	<i>16.25</i>	<i>164</i>	<i>0.07</i>	<i>11</i>
<i>162-16-10.2</i>	<i>31 May 2001</i>	<i>16.17</i>	<i>155</i>	<i>16.23</i>	<i>160</i>	<i>0.06</i>	<i>5</i>
<i>162-17-14</i>	<i>9 June 2001</i>	<i>15.99</i>	<i>122</i>	<i>16.33</i>	<i>152</i>	<i>0.34</i>	<i>30</i>

In situ data were acquired by voltammetry from the in situ electrochemical analyzer coupled to the pump profiler package (detection limits of 3 μ M for O₂ and 30 nM for H₂S). Normal typeface represents northwest stations, boldface are the west-central stations, and italicized are southwest stations.

Table 3
Comparisons of in situ voltammetry suboxic zone profiles among stations from the northwest, west-central, and southwest Black Sea

Comparison	Parameter	<i>p</i> -value
NW–WC	O ₂ disappearance (σ_t)	0.3036
	O ₂ disappearance (m)	0.1000
	H ₂ S onset (σ_t)	0.3174
	H ₂ S onset (m)	0.0693
	Suboxic thickness (σ_t)	0.3088
	Suboxic thickness (m)	0.1190
NW–SW	O ₂ disappearance (σ_t)	0.0165
	O ₂ disappearance (m)	0.0958
	H ₂ S onset (σ_t)	0.0158
	H ₂ S onset (m)	0.2805
	Suboxic thickness (σ_t)	0.0791
	Suboxic thickness (m)	0.0152
WC–SW	O ₂ disappearance (σ_t)	0.0091
	O ₂ disappearance (m)	0.0010
	H ₂ S onset (σ_t)	0.0169
	H ₂ S onset (m)	0.0003
	Suboxic thickness (σ_t)	0.0065
	Suboxic thickness (m)	0.0512

p-value results from two-sample *t* test assuming unequal variances and $\alpha = 0.05$. Significant differences are in boldface.

Particulate Mn concentrations were up to 12 times greater in the southwest versus the west-central and northwest Black Sea (Fig. 10A, C, E).

Because manganese cycling in oxic/anoxic marine systems is tightly coupled with sulfide/elemental sulfur/sulfate cycling (Luther et al., 1991; Millero,

1991), it is also important to consider elemental sulfur distributions and concentrations. MnO₂ can serve as an oxidant for H₂S after dissolved Mn²⁺ is oxidized to particulate Mn(III,IV) above the anoxic layer and sinks back into the sulfidic zone (Tebo, 1991). Mn²⁺ can catalyze the oxidation of H₂S by O₂ when Mn²⁺ and O₂ co-exist in the same water mass (Luther et al., 1991). In most of the Black Sea away from the southwest region, elemental sulfur profiles generally showed subsurface maxima (concentrations less than 150 nM) just below the particulate Mn maxima and just above the onset of sulfide, in agreement with data from 1988 (Jorgensen et al., 1991; Luther et al., 1991). Sulfide oxidation was more intense and elemental sulfur at higher concentrations (Fig. 11) in the southwest Black Sea due to lateral influx of O₂ above and below the H₂S onset. Particulate Mn was found within the sulfide zone (i.e. Fig. 10E,F compared with Fig. 8A) and it coincided with the maximum in elemental sulfur (Fig. 11), consistent with O₂ oxidation of Mn(II) to Mn(III, IV) in turn oxidizing H₂S to polysulfide and S₈. Because the reaction of MnO₂ with H₂S is fast and S₈ is measurable as shown in Table 1 (Eqs. (3a,b)), these data indicate that the Au/Hg electrode was measuring traces of soluble S₈ at the H₂S onset in the southwest Black Sea.

4. Conclusions

High-resolution voltammetric profiling coupled to techniques for measurement validation resulted

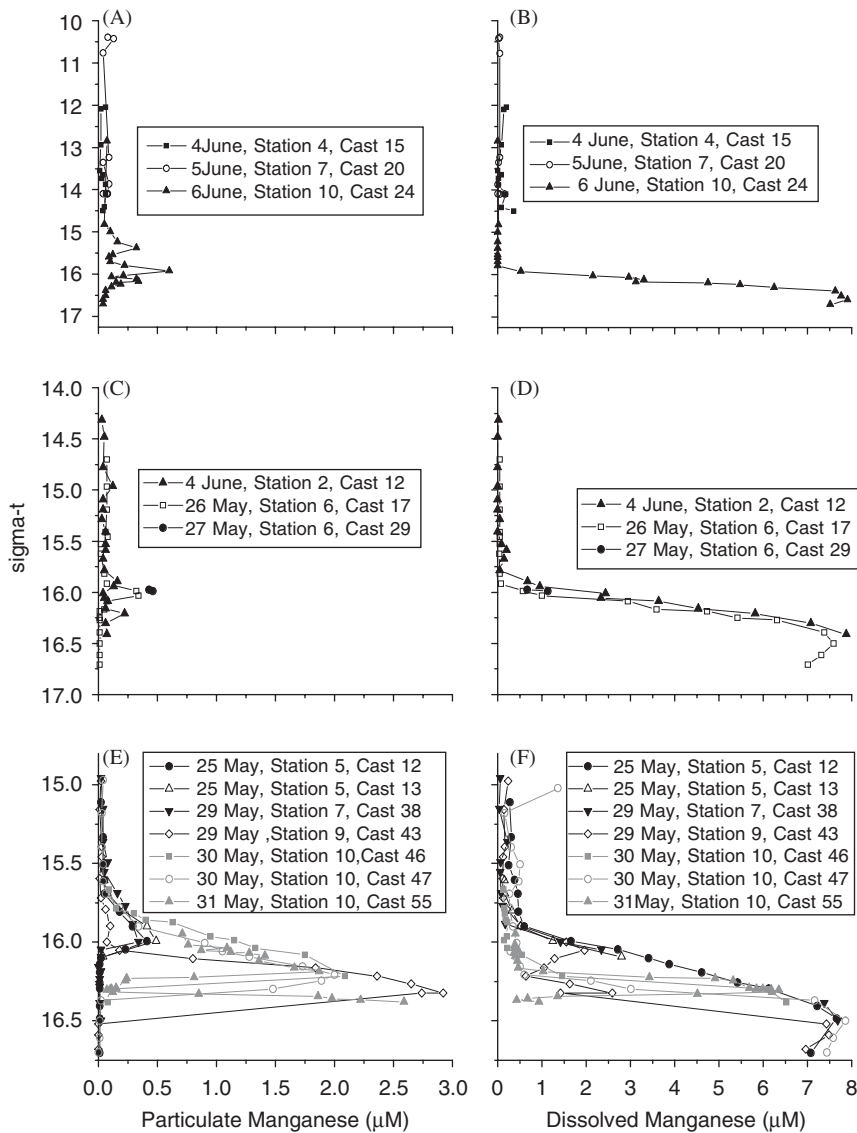


Fig. 10. A. Particulate Mn in the northwest Black Sea, B. Dissolved Mn in the northwest, C. Particulate Mn in the west-central, D. Dissolved Mn in the west-central, E. Particulate Mn in the southwest, F. Dissolved Mn in the southwest.

in an unprecedented high-resolution characterization of the oxic–suboxic–sulfidic transition throughout the western Black Sea, including measurement of partially oxidized sulfur intermediates just above onset of H_2S . Increased intensity in sulfide oxidation was observed in southwest stations as a result of oxygen-rich ventilation by the Bosphorus Plume, indicated by deep oxygen penetration (mean σ_t of 16.0 in the southwest as compared to 15.5 in the west-central and northwest) and suppression of H_2S onset (mean σ_t of 16.2 in the southwest as compared

to 16.1 in the west-central and northwest). The thickness of the suboxic zone also varied, with a significantly thinner zone in the southwest. However, at no time was sulfide observed to coexist with oxygen, indicating that an alternative oxidant must be responsible for sulfide oxidation in the Black Sea. Mn(III/IV) phases are most likely the direct oxidants of H_2S , while O_2 is the ultimate oxidant via Mn(II) catalysis (Luther et al., 1991; Millero, 1991; Tebo, 1991; Kononov and Murray, 2002).

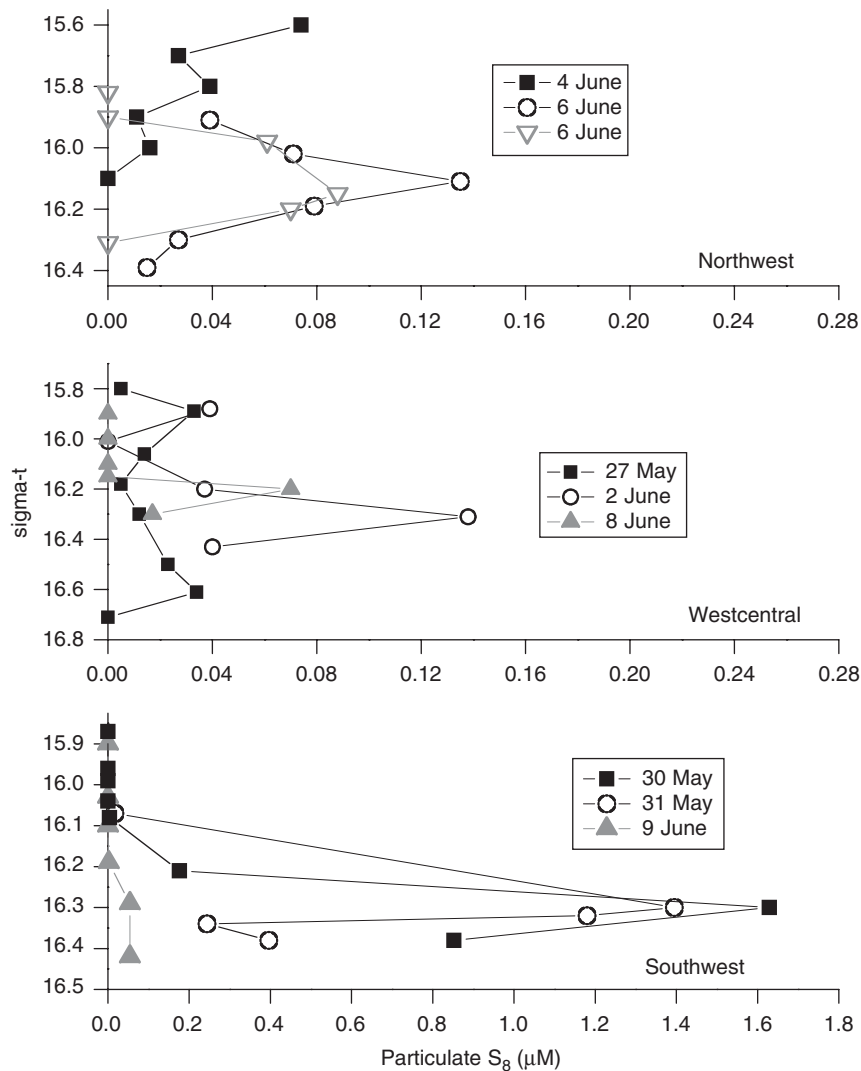


Fig. 11. Particulate S₈ in the northwest, west-central, and southwest Black Sea. Note concentration change between A, B versus C.

Acknowledgments

We thank the crew and captain of the R.V. *Knorr* for their help. This work was funded by grants from the National Science Foundation to G.W.L. (OCE-0096365), B.M.T. (OCE-0221500 and EAR-9725845), and James W. Murray (OCE-9906656), as well as the US Civilian Research and Development Foundation to S.K.K., A.S.R., and G.W.L. (UG2-2080).

References

- Anderson, L.G., Dyrssen, D., Hall, P.O.J., 1988. On the sulphur chemistry of a super-anoxic fjord, framvaren, South Norway. *Marine Chemistry* 23, 283–293.
- Arthur, M.A., Dean, W.E., 1998. Organic-matter production and preservation and evolution of anoxia in the Holocene Black Sea. *Paleoceanography* 13 (4), 395.
- Brendel, P.J., Luther III, G.W., 1995. Development of a gold amalgam voltammetric microelectrode for the determination of dissolved Fe, Mn, O₂, and S(-II) in porewaters of marine and freshwater sediments. *Environmental Science and Technology* 29 (3), 751–761.
- Brewer, P.G., Spencer, D.W., 1971. Colorimetric determination of manganese in anoxic waters. *Limnology and Oceanography* 16 (1), 107–110.
- Bull, D.C., Taillefert, M., 2001. Seasonal and topographic variations in porewaters of a southeastern USA salt marsh as revealed by voltammetric profiling. *Geochemical Transactions* 13, 173.
- Codispoti, L.A., Friederich, G.E., Murray, J.W., Sakamoto, C.M., 1991. Chemical variability in the Black Sea: implications of data obtained with a continuous vertical profiling system that penetrated the oxic–anoxic interface. *Deep Sea*

- Research Part II: Topical Studies in Oceanography 38, S691–S710.
- Faschuck, D.Y., Ayzatullin, T.A., 1986. A possible transformation of the anaerobic zone of the Black Sea. *Oceanology (Russian)* 26, 171–178.
- Ginzburg, A.I., Kostianoy, A.G., Nezhlin, N.P., Soloviev, D.M., Stanichny, S.V., 2002. Anticyclonic eddies in the northwestern Black Sea. *Journal of Marine Systems* 32 (1–3), 91–106.
- Glazer, B.T., Cary, S.C., Hohmann, L., Luther III, G.W., 2002. In situ sulfur speciation using Au/Hg microelectrodes as an aid to microbial characterization of an intertidal salt marsh microbial mat. In: Taillefert, M., Rozan, T.F. (Eds.), *Environmental Electrochemistry: Analyses of Trace Element Biogeochemistry*. American Chemical Society, Washington, DC.
- Ivanov, L.I., Samodurov, A.S., 2001. The role of lateral fluxes in ventilation of the Black Sea. *Journal of Marine Systems* 31 (1–3), 159–174.
- Jorgensen, B.B., Fossing, H., Wirsén, C., Jannasch, H., 1991. Sulfide oxidation in the anoxic Black Sea chemocline. *Deep-Sea Research Part II: Topical Studies in Oceanography* 38 (Suppl. 2), S1083–S1103.
- Konovalov, S.K., Ivanov, L.I., Samodurov, A.S., 2001. Fluxes and budget of sulphide and ammonia in the Black Sea anoxic layer. *Journal of Marine Systems* 31 (1–3), 203–216.
- Konovalov, S.K., Luther III, G.W., Friederich, G.E., Nuzzio, D.B., Tebo, B., Murray, J.W., Oguz, T., Glazer, B.T., Trouwborst, R.E., Clement, B.G., Murray, K.J., Romanov, A.S., 2003. Lateral injection of oxygen with the Bosphorus plume-fingers of oxidizing potential in the Black Sea. *Limnology and Oceanography* 48 (6), 2369–2376.
- Konovalov, S.K., Murray, J.W., 2002. Variations in the chemistry of the Black Sea on a time scale of decades (1960–1995). *Journal of Marine Systems* 31 (1–3), 217–243.
- Lane-Serff, G.F., Rohling, E.J., Bryden, H.L., Charnock, H., 1997. Postglacial connection of the Black Sea to the Mediterranean and its relation to the timing of sapropel formation. *Paleoceanography* 12 (2), 169–174.
- Latif, M.A., Ozsoy, E., Oguz, T., Unluata, U., 1991. Observations of the Mediterranean inflow into the Black Sea. *Deep Sea Research Part II: Topical Studies in Oceanography* 38 (2), S711–S723.
- Lewis, B., Landing, W., 1991. The biogeochemistry of manganese and iron in the Black Sea. *Deep Sea Research II: Topical Studies in Oceanography* 38, S773–S804.
- Luther III, G.W., Bono, A.B., Taillefert, M., 2002. A continuous flow electrochemical cell for analysis of chemical species and ions at high pressure: laboratory, shipboard and hydrothermal vent results. In: Taillefert, M., Rozan, T.F. (Eds.), *Environmental Electrochemistry: Analyses of Trace Element Biogeochemistry*. American Chemical Society, Washington, DC, pp. 54–73.
- Luther III, G.W., Church, T.M., Powell, D., 1991. Sulfur speciation and sulfide oxidation in the water column of the Black Sea. *Deep Sea Research Part II: Topical Studies in Oceanography* 38, S1121–S1138.
- Luther III, G.W., Ferdelman, T.G., 1993. Voltammetric characterization of iron (II) sulfide complexes in laboratory solutions and in marine waters and porewaters. *Environmental Science and Technology* 27, 1154–1163.
- Luther III, G.W., Giblin, A.E., Varsolona, R., 1985. Polarographic analysis of sulfur species in marine porewaters. *Limnology and Oceanography* 30 (4), 727–736.
- Luther III, G.W., Reimers, C.E., Nuzzio, D.B., Lovalvo, D., 1999. In situ deployment of voltammetric, potentiometric, and amperometric microelectrodes from a ROV to determine dissolved O₂, Mn, Fe, S(-2), and pH in porewaters. *Environmental Science and Technology* 33, 4352–4356.
- Luther III, G.W., Glazer, B.T., Hohmann, L., Popp, J.I., Taillefert, M., Rozan, T.F., Brendel, P.J., Theberge, S.M., Nuzzio, D.B., 2001a. Sulfur speciation monitored in situ with solid state gold amalgam voltammetric microelectrodes: polysulfides as a special case in sediments, microbial mats and hydrothermal vent waters. *Journal of Environmental Monitoring* 3 (1), 61–66.
- Luther III, G.W., Rozan, T.F., Taillefert, M., Nuzzio, D.B., Di Meo, C., Shank, T.M., Lutz, R.A., Cary, S.C., 2001b. Chemical speciation drives hydrothermal vent ecology. *Nature* 410 (6830), 813–816.
- Millero, F.J., 1991. The oxidation of H₂S in Black Sea waters. *Deep Sea Research Part II: Topical Studies in Oceanography* 38, S1139–S1150.
- Murray, J.W., Codispoti, L.A., Friederich, G.E., 1995. Oxidation-reduction environments: the suboxic zone in the Black Sea. In: Huang, C.P., O'Melia, C.R., Morgan, J.J. (Eds.), *Aquatic Chemistry: Interfacial and Interspecies Processes*, vol. 244. American Chemical Society, Washington, DC, pp. 157–176.
- Murray, J.W., Jannasch, H.W., Honjo, S., Anderson, R.F., Reeburgh, W.S., Top, Z., Friederich, G.E., Codispoti, L.A., Izdar, E., 1989. Unexpected changes in the oxic-anoxic interface in the Black Sea. *Nature* 338, 411–413.
- Murray, J.W., Top, Z., Ozsoy, E., 1991. Hydrographic properties and ventilation of the Black Sea. *Deep-Sea Research Part II: Topical Studies in Oceanography* 38 (Suppl. 2A), 663–689.
- Oguz, T., Latun, V.S., Latif, M.A., Vladimirov, V.V., Sur, H.I., Markov, A.A., Ozsoy, E., Kotovshchikov, B.B., Eremeev, V.V., Unluata, U., 1993. Circulation in the surface and intermediate layers of the Black Sea. *Deep Sea Research Part I: Oceanographic Research Papers* 40 (8), 1597–1612.
- Oguz, T., Rozman, L., 1991. Characteristics of the Mediterranean underflow in the southwestern Black Sea continental shelf/slope region. *Oceanology Acta* 14 (5), 433–444.
- Pers, C., Rahm, L., 2000. Changes in apparent oxygen removal in the Baltic proper deep water. *Journal of Marine Systems* 25, 421–429.
- Rozan, T.F., Luther III, G.W., 2001. An anion chromatography/ultraviolet detection method to determine nitrite, nitrate, and sulfide concentrations in saline (pore) waters. *Marine Chemistry* 77 (1), 1–6.
- Rozan, T.F., Theberge, S.M., Luther III, G.W., 2000. Quantifying elemental sulfur (S⁰), bisulfide (HS⁻) and polysulfides (S_x²⁻) using a voltammetric method. *Analytica Chimica Acta* 415 (1–2), 175–184.
- Schuf, J., De Baar, H.J.W., 1995. Rare earth element exchange through the Bosphorus: The Black Sea as a net source of REEs to the Mediterranean Sea. *Geochimica et Cosmochimica Acta* 59 (17), 3503–3509.
- Scranton, M.I., Astor, Y., Bohrer, R., Ho, T.-Y., Muller-Karger, F., 2001. Controls on temporal variability of the geochemistry

- of the deep Cariaco Basin. *Deep Sea Research Part I: Oceanographic Research Papers* 48 (7), 1605–1625.
- Sorokin, Y.I., 1972. The bacterial population and the processes of hydrogen sulfide oxidation in the Black Sea. *Journal du Conseil International Exploration de la Mer* 34, 423–454.
- Sorokin, Y.I., 1983. The Black Sea. In: Ketchum, B.H. (Ed.), *Ecosystems of the World 26: Estuaries and Enclosed Seas*. Amsterdam, Elsevier.
- Tebo, B., 1991. Manganese (II) oxidation in the suboxic zone of the Black Sea. *Deep-Sea Research Part II: Topical Studies in Oceanography* 38 (Suppl. 2), S883–S905.
- Tugrul, S., Basturk, O., Saydam, C., Yilmaz, A., 1992. Changes in the hydrochemistry of the Black Sea inferred from water density profiles. *Nature* 359, 137–139.
- Wang, F., Tessier, A., 1998. Voltammetric determination of elemental sulfur in pore waters. *Limnology and Oceanography* 43 (6), 1353–1361.