# Research

# Thiols in Coastal Waters of the Western North Sea and English Channel

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Thiols were determined in coastal waters of the western North Sea and English Channel. Detection was carried out on-board ship on-line by flow-analysis with detection by cathodic stripping voltammetry and calibration with thiourea. The thiol concentrations ranged from 0.70 to 3.60 nM (thiourea equivalents) and were found to vary over a relatively short distance perpendicular to the coast. Low concentrations in the area of greatest estuarine input (the Humber-Wash area) indicated that the thiols did not originate from low-salinity waters. Instead, variations in the thiol concentration were found to parallel those of chlorophyll. This correlation was confirmed by depth profiles which showed a trend for the thiols similar to that of chlorophyll. The data demonstrates that thiols are more widespread than anticipated, and that marine phytoplankton is an important source of the thiols. In view of their known ability to bind with metals, these data indicate that the thiols could be an important candidate to act as such ligands in the marine system. The measurements gave no evidence for the presence of sulfide in these waters which means that it must be present at less than 20% of the detected thiol levels.

## Introduction

Transformations between the various forms of inorganic and organic sulfur compounds are key factors in the global sulfur cycle $(1-4)$. $SO_4^{2-}$ is the predominant form of inorganic sulfur in the presence of dissolved oxygen, which can be assimilated by marine organisms and subsequently reduced to organic sulfur containing compounds. Sulfide is the predominant species in reduced conditions, but it is unstable with respect to oxidation by hydrogen peroxide and iodate $(5, 6)$ in seawater. Nevertheless, it has been found to occur, at low concentrations (pico to nanomolar), in aerobic conditions $(7, 8)$, presumably stabilized by complexation with metals in addition to in-situ biological production. Similarities in the depth profiles of sulfide and chlorophyll suggest a biological origin for the sulfide $(9)$.

COS (carbonyl sulfide) is present in seawater at pico to nanomolar concentrations $(10, 11)$, with diel variations in surface waters thought to be due photochemical reactions $(10)$. A photosensitized reaction of organic sulfur compounds including thiols is a source for COS $(12)$. The spatial variability (i.e., offshore and nearshore) in the COS distribution is associated with the distribution of organic compounds which are photolyzed to COS, and this could be the reason the concentration of COS in the coastal waters is higher than in open seawater $(3)$. Thiols are therefore a source of COS and subsequently of sulfide, mediated by photochemical and biological reactions.

Thiols, like sulfide, are important ligands for metals. These compounds could be a major ligand for copper in seawater in view of their high complex stability with that metal $(13)$. Glutathione is thought to be the most abundant nonprotein thiol in animals, plants, and various bacteria (typical concentrations range between 0.1 and 10 mM) $(14, 15)$. Nanomolar levels of *particulate* glutathione are known to exist in coastal waters, co-varying with chlorophyll $(16)$, and *dissolved* glutathione has been shown to occur in the water column of the Atlantic $(17)$.

Sulfide in seawater can be determined after $H_2S$ volatilisation from acidified samples with cryogenic trapping followed by detection by gas chromatography with photo-ionization detection $(7)$ or with flame-photometric detection $(18, 19)$ or by cathodic stripping voltammetry $(8)$, which involves a reaction of electrochemically labile sulfide (comprising free sulfide and sulfide released from complex dissociation in the diffusion layer) with a mercury electrode. The voltammetric method has not been compared to the GC methods, but oceanic concentrations can be compared nevertheless assuming oceanographic consistency: the GC methods have shown oceanic total sulfide concentrations in the upper water column on the order of 100 pM, with very low free sulfide concentrations, below 5 pM $(19)$. Higher total sulfide levels have typically been found by the CSV method: typically 2 nM in the oceanic water column of unknown sulfide species $(8)$. Our preliminary work has demonstrated that although CSV can be used to determine sulfide, this has to be done using a flow-cell because free sulfide, as well as sulfide complexed with certain trace metals, gives an unstable peak which rapidly decreases due to the formation of an insoluble product with mercuric ions from the mercury electrode (present as residues in the voltammetric cell), whereas a stable peak is obtained for various thiol compounds $(20)$. This preliminary work suggested the possibility that the at least part of the stable sulfide-like voltammetric peak in seawater may be due to thiol compounds rather than to sulfide.

Here voltammetry is used to obtain thiol concentrations, and check for the presence of sulfide, in coastal water of the western North Sea and English Channel. Flow-analysis was used with detection by CSV (FA-CSV), and the measurements were on-line, in water pumped on-board the ship. Separate measurements using a conventional voltammetric cell were made to confirm whether the peak was stable and due to sulfide or thiols. A data set was obtained of some 7000 thiol measurements in these waters. These data were used to determine relationships of thiols to various other parameters (e.g., salinity, temperature, chlorophyll). Automated volta-mmetric methods, on board ship, have been used before for in-line monitoring of trace metals in surface and coastal waters of the western North Sea $(21, 22)$ but not for sulfide or thiol compounds.

## Materials and Methods

**Equipment.** Water was pumped at a rate of 4 L min$^{-1}$ from a depth of $\sim$4 m on-board ship using a peristaltic pump via

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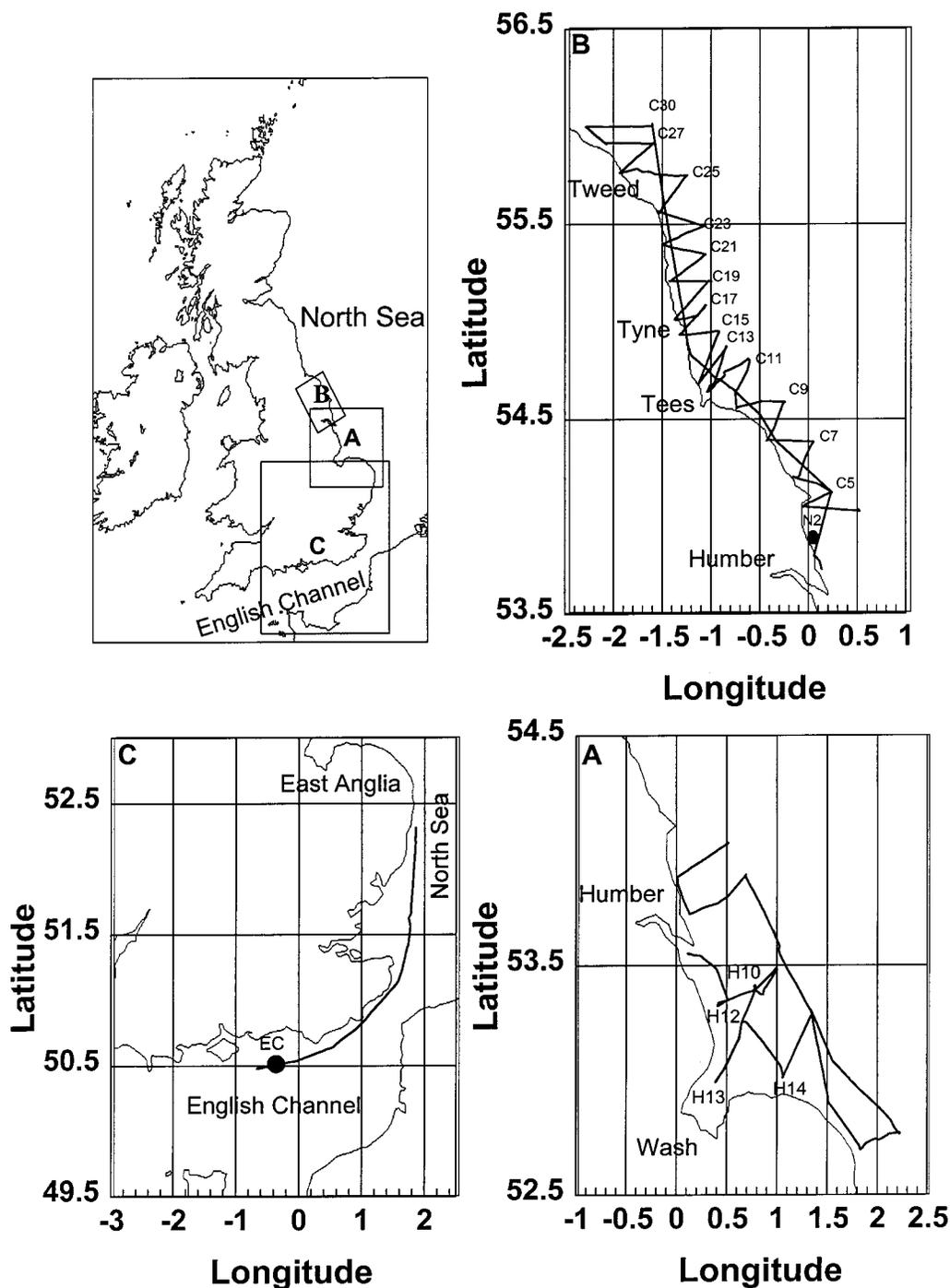
FIGURE 1. Study area of the North Sea Cruise. (A) Humber–Wash grid including the Humber, Wash, and part of the East Anglia; (B) northeast coastal grid between the Humber and the Tweed rivers; (C) East Anglia-English Channel grid.

a 20 m poly(vinyl chloride) (PVC) hose of 12 mm internal diameter. To hold the hose under water, the hose was attached to a heavy (~50 kg), epoxy-coated, iron, "fish". The "fish" was suspended to the left of the bow of the ship at 3 m depth. The distance between the "fish" and the hull was approximately 3–4 m. A high density polyethylene funnel was attached to the front of the "fish", connected to the inlet of the hose. In-line filtration of the seawater was achieved using a homemade filtration unit which was flushed continuously in a cross-flow fashion: the seawater was flowing tangentially along a 0.45 μm membrane filter in a Swinnex 47 filter holder (Millipore part SX00 047 00) which was fixed on the side of the hose and cut to allow the water to pass directly along the filter.

Salinity, transmissometry, temperature, chlorophyll, nitrate, nitrite, and phosphate data were obtained from the British Oceanographic Data Centre (BODC) in Bidston. The first four parameters were determined by members of the Proudman Oceanography Laboratory (POL) and nitrate was measured by the Plymouth Marine Laboratories (PML). Chlorophyll was determined on-line using a fluorimeter calibrated using chlorophyll standard.

Discrete samples from the water column of the North Sea and the English Channel were collected using a CTD-rosette sampler with modified precleaned 10 liter FEP-coated Go-Flo bottles, filtered and measured by FA-CSV. For reasons of comparison, filtered samples were collected using the in-line pumping system and were measured on-board ship by

FA-CSV as well as batch-mode CSV. All discrete samples were analyzed in duplicate. A stock solution of 0.1 M thiourea (BDH) was prepared in Milli-Q water.

**Voltammetric Procedure To Detect Thiol Compounds.** Voltammograms were recorded using an Autolab voltammeter (Eco Chemie, Netherlands) and a hanging mercury drop electrode (HMDE) (663 VA stand, Metrohm, Switzerland). The voltammetric cell was replaced by a homemade, Perspex, flow-cell and in-line purging system to remove oxygen (20). The nozzle of the jet of the flow-cell was adjusted to within ~1 mm of the mercury drop. The water level in the cell was maintained constant by continuously pumping water out via a Teflon tube fixed at the water surface. The system was controlled by an IBM-compatible computer. The software (EASVDB) was adapted from the original software (EAS from Eco Chemie) to enable continuous on-line analysis. A square-wave modulation (150 Hz) was applied to the CSV scans with a pulse-height of 25 mV and step height of 2.4 mV. Potentials were set relative to a double-junction, Ag/AgCl, saturated AgCl, 3 M KCl, reference electrode, with a salt-bridge filled with 3 M KCl. Purified nitrogen gas was used to flush the container holding the semipermeable tubing for oxygen removal. The residence time of the seawater in the degassing unit was 20 s.

The following voltammetric parameters were used: the deposition potential was set to $-0.05$ V; four mercury drops were discarded before a new mercury drop was extruded; a deposition time of 30 s was used during which water was pumped through the flow-cell and the bulk solution was purged. A 3 s quiescent period was allowed before the potential scan was initiated at $-0.2$ V and terminated at $-0.7$. The precision of individual shipboard measurements was $\pm5\%$. The system was intermittently calibrated at $6-8$ h intervals. The sensitivity was calibrated using a freshly collected, discrete, sample in a 250 mL polyethylene bottle, and a second one to which 2.5 nM thiourea had been added. The sensitivity was calculated from the difference of the average peak heights of the two samples (20 scans for each sample). Thiol concentrations are expressed in nM equivalents of thiourea.

**Study Area.** Figure 1 shows the study area and cruise track (RRS Challenger, $1-13$ July, 1995) for the North Sea and the English Channel. The area investigated was divided into three major regions: (1) Humber–Wash grid starting from the north of the Humber Estuary going south to 52.8° N; this followed a box-like pattern (Figure 1A); (2) northeast coastal grid where the ship followed a zigzag pattern starting from the north of the Humber Estuary and going north to Tweed (Figure 1B) and then back parallel to the coast; and (3) the East Anglia-English Channel grid where the ship steamed parallel to the coast from the south of East Anglia along the southwest of the North Sea to the northern side of the English Channel (Figure 1C). This cruise was part of the Land Ocean Interaction Study (LOIS) project of the National Environmental Research Council (NERC).

## Results

**Verification of the Identity of the Thiol/Sulfide Peak in Seawater.** Voltammetry of seawater collected during the RRS Challenger cruise revealed the presence of a sulfide or thiol-type peak at $-0.54$ V (Figure 2). Batch-mode voltammetry of several (10) filtered samples was used on-board ship to verify whether the response was due to sulfide or thiol compounds. The voltammetric response was stable for a period of at least 10 min, indicating that the response was not due to sulfide [which would have caused a rapidly decreasing response but to thiol compounds (20)]. The decrease of the sulfide peak had been ascribed before to degassing of H₂S (23) and to changes in the sample composition (24); however, in addition to those reactions it has been demonstrated that
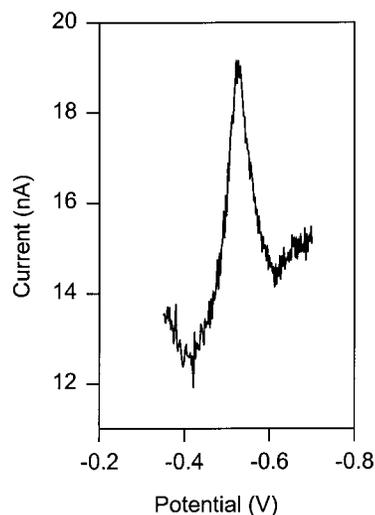
FIGURE 2. Voltammetric response of sulfide/thiol compounds in North Seawater.

TABLE 1. Comparative Measurements of Thiol Concentrations (nM thiourea equivalents) by Flow and Batch-Mode Voltammetry On-board Ship during a Challenger Cruise (CH119C) on the North Sea in Samples Taken at CTD-Stations

| station | batch analysis | flow analysis |
|---|---|---|
| H12 | 1.51 | 1.64 |
| H13 | 1.70 | 1.72 |
| H18 | 1.77 | 1.80 |
| C5 | 1.52 | 1.61 |
| C10 | 1.80 | 1.96 |
| C12 | 3.12 | 2.99 |
| C18 | 2.37 | 2.36 |
| C21 | 1.65 | 1.71 |
| C24 | 1.90 | 1.73 |
| C29 | 1.86 | 1.91 |

the reaction with mercury waste is sufficient to rapidly remove the sulfide contribution to this peak (20).

Several filtered samples of surface water were analyzed on-board ship at selected CTD stations to compare analyses by flow and batch-mode voltammetry (Table 1). Stable peak heights of the batch-mode measurements in all tested samples demonstrated that the sulfide concentration was negligible compared to that of the thiols. In view of an average standard deviation of 5% of successive scans in the batch cell, the sulfide concentration amounted to less than 5% of that of the thiols. This means that the sulfide levels in these coastal waters were less than $0.05-0.15$ nM, in line with expectation for oceanic sulfide levels (9), but are somewhat less than levels of $0.1-1$ nM of total sulfide (including metal sulfide complexes) found previously in coastal and surface ocean waters (7).

Another candidate for the identity of the thiol-type peak is elemental sulfur (25): it is unlikely that a poorly soluble element like sulfur would occur in natural waters, but in any case this peak decreases rapidly (20) [confirmed in separate experiments (Laglera and van den Berg, unpublished data)] just like the sulfide peak, indicating that its contribution (if any) to the stable peak used here to detect thiols, was negligible.

The sulfide and thiols detected by voltammetry are free concentrations: complexation by trace metals could conceivably mask major amounts of these compounds, as only the free sulfide and thiol concentrations are detected by the voltammetric method, possibly augmented by dissociation of some of the complexed fraction. At the nanomolar levels
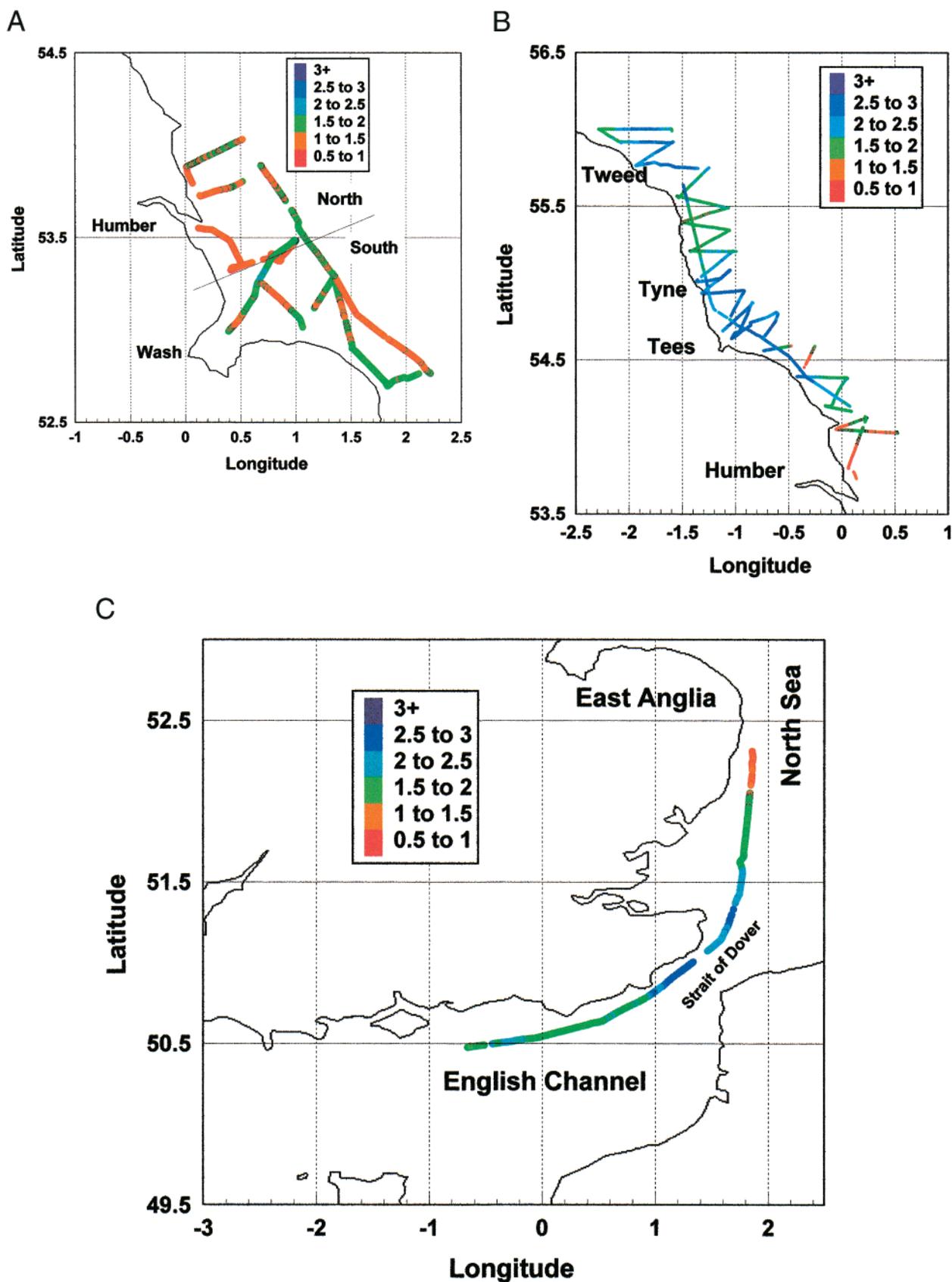
FIGURE 3. Distribution of thiols along the North Sea coast and the Channel in the cruise track. Colors on the map indicate detected thiol concentrations. (A) the Humber—Wash region. (B) The East Anglia-English Channel region. (C) The Channel.

of trace metals in the coastal waters studied here only copper would be likely to complex sulfide as the complexes with other metals such as cadmium, lead, aluminum, and others are too weak to obtain significant complexation at low nanomolar levels. The stability of copper complexes with sulfide [log $K_{CuHS}$ = 12.9 (26)] is similar to that for organic
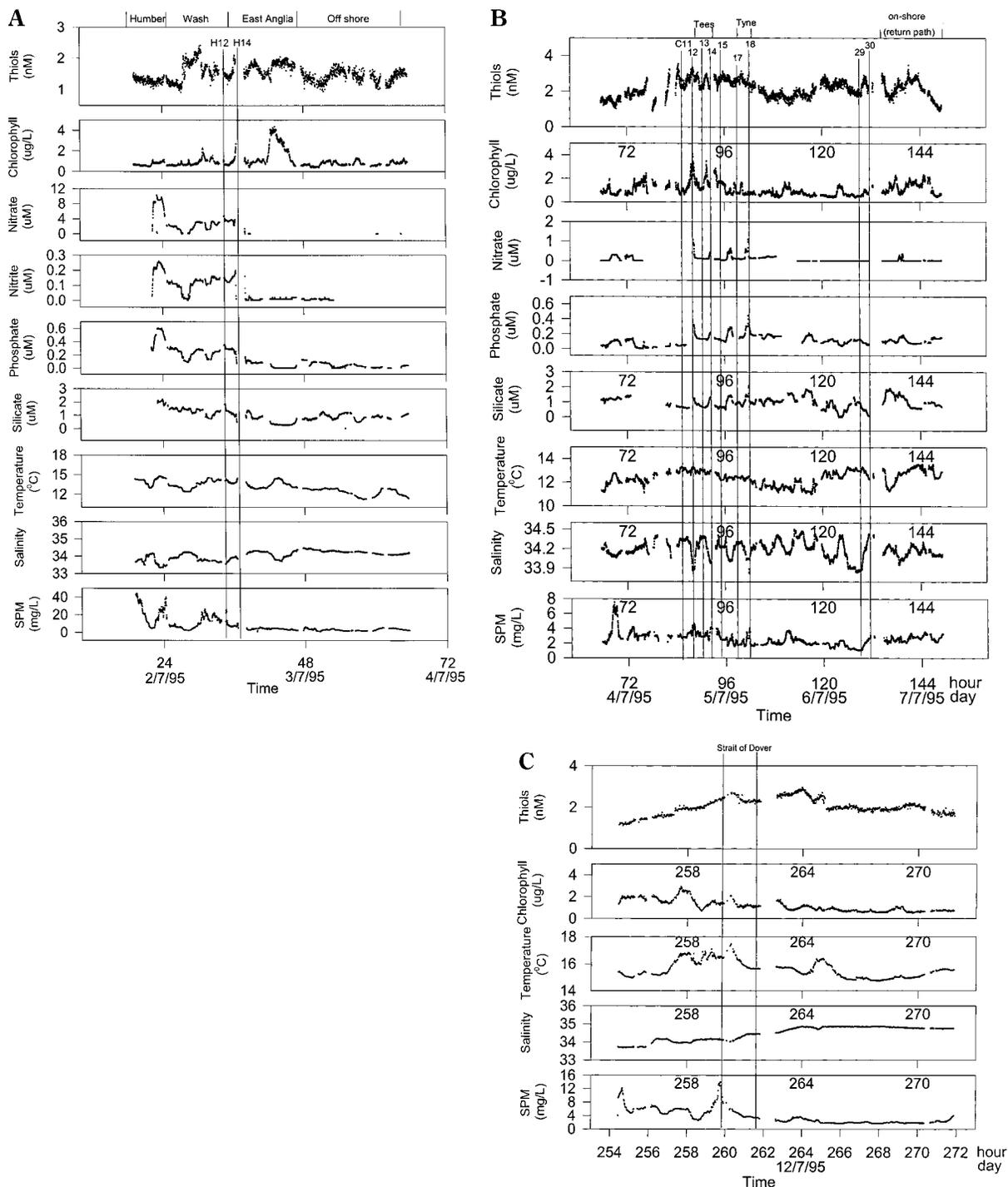
FIGURE 4. Concentrations of thiols and other parameters as a function of time in U.K. coastal waters. Vertical lines illustrate the areas where a good correlation was found between thiols and chlorophyll. (A) The Humber−Wash region. (B) The north east coastal region. (C) The East Anglia-English Channel region.

copper complexes in seawater (27, 28); the organic ligands occur at greater levels and in excess in coastal waters, suggesting that these would tend to outcompete low sulfide levels leaving a major proportion of the sulfide free; nevertheless, it is possible that a significant fraction of sulfide is masked by copper. Similarly, the detected thiol concentrations are free thiol concentrations as the thiol peak is lowered by complexation with copper (13). For instance glutathione forms very stable complexes with copper, $\log K'_{CuGS} = 12.4$, similar to sulfide, and its free concentration could therefore be lowered by complexation with copper.

The CSV sensitivity was calibrated using thiourea which along with several thiol compounds (like glutathione and thioacetamide) gives a peak indistinguishable from that apparent in the seawater (20); thiourea was selected for practical reasons as it gives a stable peak with good sensitivity, and the stock solutions are easy to prepare; its selection does not indicate that it is a candidate for the identity of the thiol peak as it is not clear whether any thiourea occurs naturally in the environment at all. At present, we do not know which thiol compound is actually responsible for the thiol peak so in principle any thiol compound could be used for this
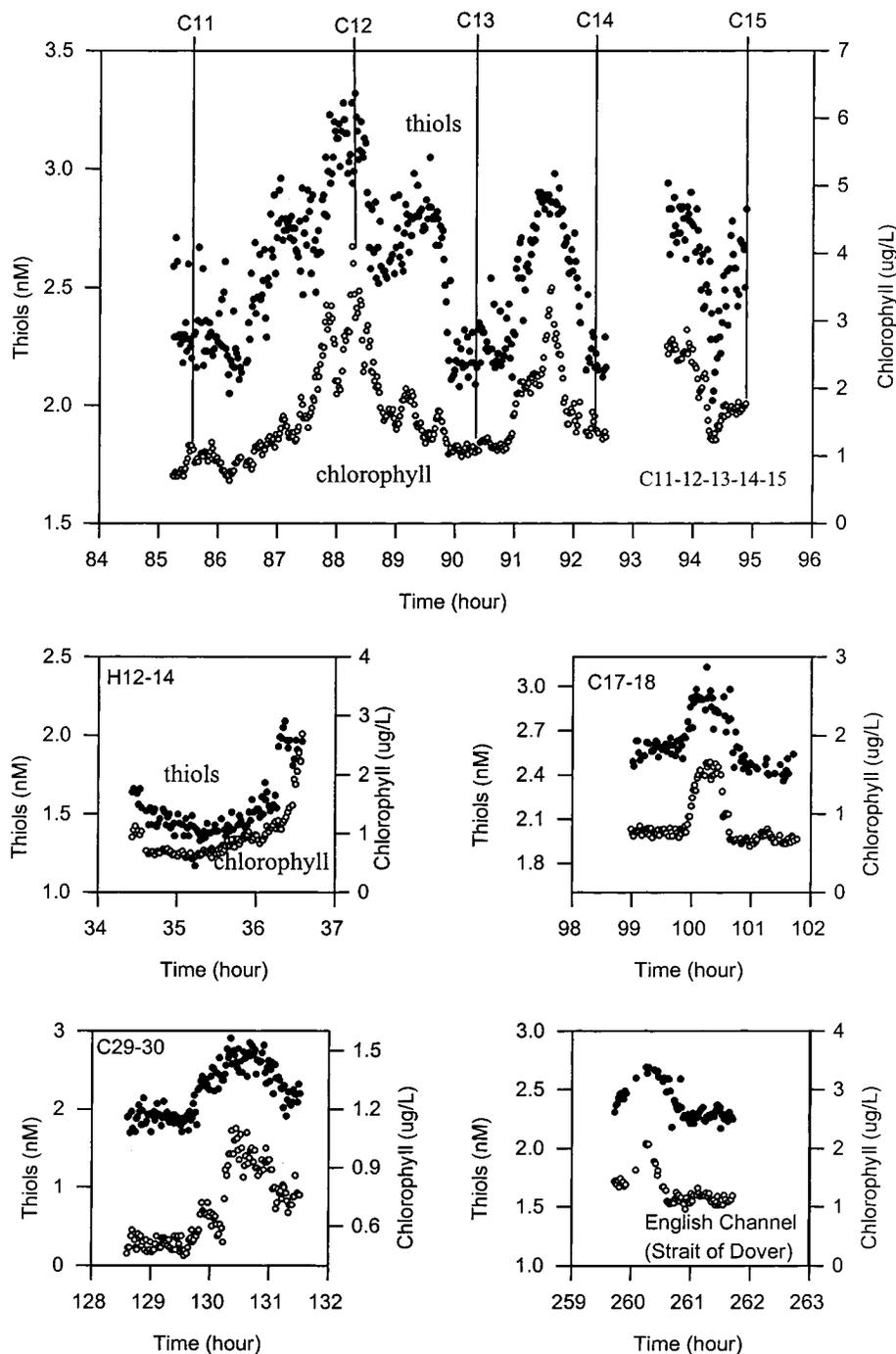
FIGURE 5. Comparison of thiol and chlorophyll concentrations of selected areas during RRS Challenger cruise to the North Sea.

calibration; however, glutathione is abundant in marine microorganisms which makes it a prime candidate (17). Sulfide and sulfur could not be used as calibrant as their peak is highly unstable in the batch cell due rapid reaction with mercury traces.

**Thiols in the Western North Sea and the English Channel.** Thiol concentrations in North Sea and Channel waters in the cruise track of the ship are shown in Figure 3. Concentrations are indicated using colors. A total of 7030 data was collected throughout the cruise at a rate of 50 measurements/h. The thiol concentrations were found to range from 0.77 to 3.54 nM with a mean of 1.81 ± 0.54 nM ($n$ = 7030) (all thiol concentrations are given as thiourea equivalents on the nanomolar scale). Highest concentrations were found in areas adjacent to the Tees-Tyne, the Tweed

(North east coastal region), and the narrow part of the Strait of Dover (East Anglia-English Channel region). The Humber—Wash region (with the greatest estuarine input of low salinity waters) was characterized by lower thiol concentrations.

Previous voltammetric studies utilizing the same voltammetric peak interpreted it as being due to sulfide. Thus, concentrations of 10—50 nM sulfide-like substances were reported in the northern Adriatic (29) which is known for large algal blooms, and 0.1—2.0 nM in the north-western Atlantic (8). Those studies used calibration with sulfide.

Cross calibration of our equipment with thiourea and sulfide showed that each nanomole of thiourea yielded a voltammetric response equivalent to 2.76 nmol of sulfide. The Adriatic data are therefore equivalent to 3.6—18 nM thiourea, and the Atlantic data are equivalent to 0.04—0.7
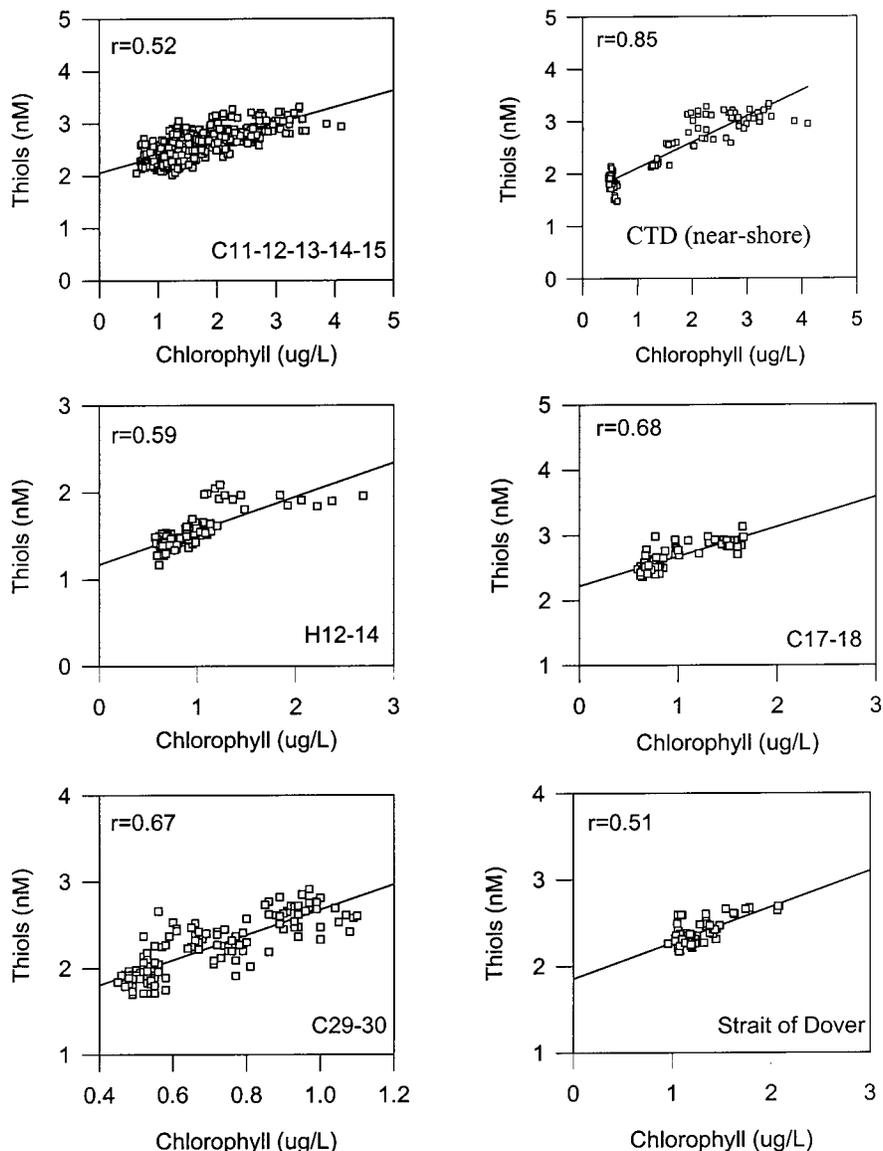
FIGURE 6. Thiols as a function of chlorophyll in selected areas of the coastal waters in the North Sea.

nM thiourea. The North sea and Channel thiol concentrations lie between these two levels which makes oceanographic sense as they might be expected to be lower in open ocean waters and higher in warm, productive, coastal waters.

The distribution of the thiols and its relation to other parameters [temperature, salinity, chlorophyll, nitrate, nitrite, phosphate, silicate, and suspended particulate material (SPM)] in the three regions of the cruise will be discussed in detail below.

**Humber-Wash Grid.** The thiol concentrations ranged between 0.92 nM and 2.41 nM, with a mean of $1.51 \pm 0.26$ ($n = 1867$). There is no systematic, clear, regional trend in the thiol distribution. The southern part of the Humber–Wash area has a mean concentration of $1.62 \pm 0.26$ ($n = 969$), which is slightly higher than that in the north ($1.39 \pm 0.21$, $n = 898$), but it is an arbitrary definition, and the difference is small. Highest concentrations were found in the Wash area near station H12 between stations H10 and H13 and near the coast of East Anglia.

**Northeast Coastal Grid.** In this part of the study, the track followed a zigzag pattern 20 mi. to and from the coast. The distribution of the thiols along the north east coast is shown in Figure 3B. The thiol concentrations varied over several nanomolar over relatively short distances due to the ship

traveling to and from the coast. The thiol concentration range was $0.88-3.54$ nM, with a mean of $2.14 \pm 0.48$ ($n = 5179$). Highest levels occurred in the Tees-Tyne area, and lowest in the most southerly part (Humber to station 5). Possible reasons for the variations become apparent when all data are compared with other parameters below.

**East Anglia-English Channel Grid.** The thiol distribution along the coast of East Anglia and through the English Channel region are shown in Figure 3C. The thiol concentrations ranged from 1.12 to 2.95 nM, with a mean of 2.04 $\pm 0.39$ ($n = 1867$). Highest concentrations were evident in the Strait of Dover.

## Discussion

**Abundance of Thiols Compared to Sulfide.** This study indicates that thiols are ubiquitous in shelf and coastal waters, at concentrations much greater than sulfide. The predominance of thiols over sulfide demonstrates the importance of taking thiols into account when voltammetry is used to detect low sulfide levels in oxygenated surface waters as the peaks are indiscernible from each other in a square-wave CSV scan. There are electrochemical differences between sulfide and sulfur (*30*) and various thiol compounds (*20*) so by varying an electrochemical parameter like the deposition potential
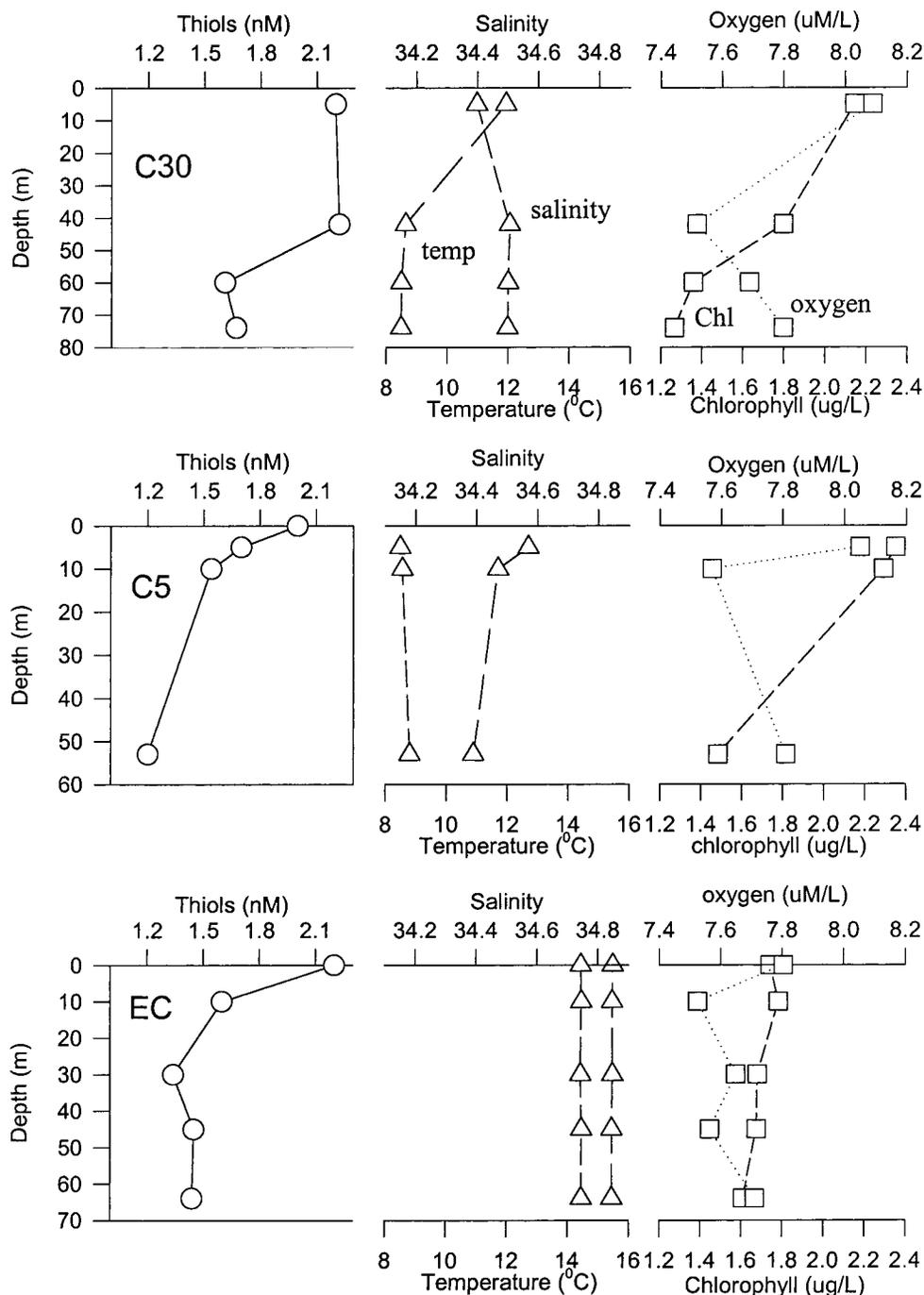
**FIGURE 7.** Depth profiles of thiols (●), temperature (△), salinity (▲), chlorophyll (□), and oxygen (■) for CTD stations during the RRS Challenger cruise to the North Sea.

it may be possible to come to a closer characterization of the thiol peak; however, the most simple characterization between sulfide, sulfur, and thiols is the instability (decreasing with subsequent scans) of the sulfide/sulfur peak in conventional (batchwise) CSV. It is possible that greater total sulfide levels are present than estimated here as the electroactive sulfide peak is lowered by complexation with metals and especially by copper (26, 31); however, such complexed sulfide (if present) does not contribute significantly to the CSV peak used here to estimate the thiol concentrations.

Interestingly, the thiol peak is also lowered by metal complexation as evidenced by copper additions to thiol solutions (32), so it is possible that the total thiol concentration is greater than indicated. Organic copper complexing ligands (of which the thiols could be an important compo-

nent) tend to be present in large excess in coastal waters (33); therefore, it is possible that the thiols are largely free and the underestimate is relatively small; this can be investigated in further work by determining the thiols concentration in the presence of a competing ligand to free up any complexed thiols.

**Relationships between Thiols and Other Parameters.** Thiol concentrations can be compared with data for chlorophyll, nitrite, nitrate, phosphate, silicate, temperature, salinity, and SPM, as a function of the cruising time in the three main regions (Humber−Wash, north east coast and east Anglia-English Channel) in Figure 4. Variability in the Humber−Wash and north east coast was particularly pronounced because of the zigzag or box-like pattern of the cruise track, whereas the variability in the east Anglia-English

Channel area was less pronounced because the cruise track was here parallel to the coast. Where the ship followed a track which zigzagged to and from the coast, the salinity can be seen to decrease and increase with this pattern. The origin of species dissolved in coastal waters can usually be evaluated by comparison with salinity: a decrease with increasing salinity would then suggest a coastal origin. However, the thiol data do not show a systematic co-variation with the salinity indicating that their distribution is not related to a specific freshwater or oceanic source. Occasionally an increase in the thiol concentration was associated with decreasing salinity and increasing nutrient concentrations (C12), but sometimes the opposite was the case (C14 and C18).

Comparison of the correlation coefficients ($r$) between thiols and the other parameters showed best correlation with chlorophyll. The correlation coefficient with chlorophyll was small (0.09) when all data were used, but significant correlations were obtained for individual sites indicating that locally the thiols co-varied with chlorophyll. Examples of the co-variation of thiol and chlorophyll are shown expanded in Figure 5, and plots of thiol against chlorophyll are shown in Figure 6. The co-variation between chlorophyll and the thiols suggest that in these waters the main origin of the thiols is from phytoplankton.

Thiol and chlorophyll concentrations obtained at CTD stations at the way-points of the cruise track (when the ship was stationary) showed much stronger correlation at the near-shore stations than at the off-shore stations: the correlation coefficient of thiols and chlorophyll for the near-shore data was 0.85, whereas it was 0.18 for the off-shore data. It is likely that the stronger trend in chlorophyll and thiol concentrations in the near-shore waters is the cause for the stronger co-variation of these data.

**Depth Profiles.** The coastal distribution suggests that marine phytoplankton plays a major role in the distribution of thiols. It is not ruled out that other processes (e.g., atmospheric input, in situ degradation of settled phytoplankton biomass and efflux of thiols across the sediment–water interface) could also affect the vertical distribution of thiols. Therefore, the vertical distribution of thiols in the North Sea was determined to verify the importance of such processes.

The depth distribution of thiols, temperature, salinity, oxygen, and chlorophyll at three stations (C30, C5, and EC) is shown in Figure 7. These stations reflect different locations and hydrographic conditions (see Figure 1). Stratification is weak at all stations due to the tidal mixing of these shallow waters. However, some stratification is apparent at station C30; it was less at C5 and absent at station EC. Generally, highest thiol concentrations (around 2.1 nM) were found in the surface waters while the concentrations decreased with depth to around 1.5 nM at all stations. The decrease in the thiol concentrations occurred at a depth of 60 m at station C30, whereas the decrease was more gradual over the top 20 m at the other two stations. The water column chlorophyll concentration also increased toward the surface causing its trend to be similar to that of the thiols. The surface increase of the thiols could be due to releases by phytoplankton with or without photochemical inducement.

Stations C30 and EC showed a small increase near the sediments which could be attributable to a release from porewaters, but the increase was small, much less than that in the surface waters. Marine porewaters and sediments are known to contain a variety of organic sulfur compounds (36–41). The interactions between hydrogen sulfide and organic compounds via Michael addition are thought to play a major role in the production of thiols in marine sediments (2, 42, 43). The deeper waters of the North Seawater column show little evidence for releases from the porewaters but it is possible that the general background level of 1.5 nM may reflect porewater releases, evidence for the trend being removed by tidal mixing. The thiol compounds in these coastal waters may therefore originate from porewaters or from in-situ production by microorganisms, or both.

The known ability of thiols to bind metals, in particular copper, suggests that the abundance of thiols in coastal waters could represent an important source of metal complexing ligands in the marine system.

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