ELSEVIER

Marine Chemistry 68 (2000) 295–306

MARINE CHEMISTRY

www.elsevier.nl/locate/marchem

Zinc speciation in the Northeastern Atlantic Ocean

Michael J. Ellwood, Constant M.G. Van den Berg *

Oceanography Laboratories, Department of Earth Science, University of Liverpool, Bedford Street North, Box 147, Liverpool L69 3BX, UK

Received 5 April 1999; accepted 23 July 1999

Abstract

Measurements of zinc and zinc complexation by natural organic ligands in the northeastern part of the Atlantic Ocean were made using cathodic stripping voltammetry with ligand competition. Total zinc concentrations ranged from 0.3 nM in surface waters to 2 nM at 2000 m for open-ocean waters, whilst nearer the English coast, zinc concentrations reached 1.5 nM in the upper water column. In open-ocean waters zinc speciation was dominated by complexation to a natural organic ligand with conditional stability constant ($\log K'_{ZnL}$) ranging between 10.0 and 10.5 and with ligand concentrations ranging between 0.4 and 2.5 nM. The ligand was found to be uniformly distributed throughout the water column even though zinc concentrations increased with depth. Organic ligand concentrations measured in this study are similar to those published for the North Pacific. However the $\log K'_{ZnL}$ values for the North Atlantic are almost and order of magnitude lower than those reported by Bruland [Bruland, K.W., 1989. Complexation of zinc by natural organic-ligands in the central North Pacific. Limnol. Oceanogr., 34, 269–285.] using anodic stripping voltammetry for the North Pacific. Free zinc ion concentrations were low in open-ocean waters (6–20 pM) but are not low enough to limit growth of a typical oceanic species of phytoplankton.

Keywords: zinc speciation; Atlantic Ocean; organic ligands; cathodic stripping voltammetry

1. Introduction

Zinc is present in nearly 300 enzymes that perform many different metabolic functions in organisms (Vallee and Auld, 1990). Concentrations of zinc in surface waters of the open ocean can be as low as 0.1 nM, (Bruland et al., 1978; Martin et al., 1993). Over the past two decades there has been increasing evidence to suggest that such low dissolved zinc concentrations may limit phytoplankton growth and their ability to fix CO_2 from seawater via the enzyme carbonic anhydrase (Anderson et al., 1978; Sunda and Huntsman, 1992; Morel et al., 1994).

Little is known about the organic complexation of zinc in open-ocean waters. Two major studies that have investigated zinc complexation in open-ocean waters have only been conducted in the North Pacific Ocean (Bruland, 1989; Donat and Bruland, 1990). The results from these two studies revealed that more than 98% of zinc in North Pacific surface waters is strongly complexed by an unidentified organic ligand. Actual free zinc ion (Zn^{2+}) concentrations range from 1 to 10 pM is surface waters which is sufficient to limit growth in some coastal species of phytoplankton in metal buffered cultures (Brand et al., 1983; Sunda and Huntsman, 1992).

To date, no open-ocean zinc speciation data has been published for the North Atlantic Ocean. Zinc concentrations in surface waters of this region range

* Corresponding author. Tel.: +44-151-794-4096; Fax: +44-151-794-4099; E-mail: sn35@liv.ac.uk

0304-4203/00/$ - see front matter
PII: S0304-4203(99)00085-7

from 0.106 to 0.3 nM, which is comparable to those in the North Pacific (Bruland and Franks, 1983; Martin et al., 1993). The North Atlantic is characterised by very high inputs of zinc from the atmosphere compared to other ocean basins (Nriagu, 1989; Duce et al., 1991; Candelone et al., 1995; Hong et al., 1997), which may influence phytoplankton growth and zinc speciation for this region assuming that the natural ligand binding zinc is produced by phytoplankton (Bruland, 1989). The aim of this study is to determine the extent of zinc complexation in the North Atlantic Ocean and the effect this has on Zn^{2+} concentrations.

1.1. Theory

The underlying theory of determining zinc speciation in seawater using cathodic stripping voltammetry (CSV) with ligand competition has been described in detail (Van den Berg, 1985; Donat and Bruland, 1990). Briefly, the complexation of zinc in seawater by a natural ligand (L) can be defined as

$$K'_{\mathrm{ZnL}} = [\mathrm{ZnL}]/([\mathrm{Zn}^{2+}][L']) \quad (1)$$

where K'_{ZnL} is the conditional stability constant of the zinc complex in seawater. $[L']$ is the concentration of L not complexed by zinc, [ZnL] is the concentration of zinc complexed with the ligand L, and $[Zn^{2+}]$ is the free zinc ion concentration. The total ligand concentration (C_{L}) is defined as

$$C_{\mathrm{L}} = [\mathrm{ZnL}] + [L'] \quad (2)$$

Substitution for $[L']$ in Eq. (1) using Eq. (2) and rearranging gives (Ruzic, 1982; Van den Berg, 1982)

$$[\mathrm{Zn}^{2+}]/[\mathrm{ZnL}] = [\mathrm{Zn}^{2+}]/C_{\mathrm{L}} + 1/(K'_{\mathrm{ZnL}}C_{\mathrm{L}}) \quad (3)$$

When values of $[Zn^{2+}]/[ZnL]$ are plotted against corresponding values of $[Zn^{2+}]$ a linear relationship is usually obtained with a slope equal to $1/C_{\mathrm{L}}$ and with the intercept yielding $1/(K'_{\mathrm{ZnL}}C_{\mathrm{L}})$. In solution, the Zn^{2+} concentration is related to the current (i_{p}) measured at the hanging mercury drop by

$$[\mathrm{Zn}^{2+}] = i_{\mathrm{p}}/(S\alpha') \quad (4)$$

where S is the sensitivity (peak current/zinc concentration) of the system. S is usually estimated from the linear portion of the titration curve after effectively all of L has been titrated with zinc. α' is the overall side-reaction coefficient for zinc

$$\alpha' = \alpha_{\mathrm{Zn}} + \alpha_{\mathrm{ZnPDC}} \quad (5)$$

where α_{Zn} is the inorganic side-reaction coefficient for zinc, which was calculated to be 2.1 using an ion-pairing model and with constants from Turner et al. (1981). α_{ZnPDC} is the side-reaction coefficient for zinc complexed with the added competing ligand pyrrolidinedithiocarbamate (PDC). In solution α_{ZnPDC} is fixed by the amount of PDC added

$$\alpha_{\mathrm{ZnPDC}} = K'_{\mathrm{ZnPDC}}[\mathrm{PDC}'] \quad (6)$$

where K'_{ZnPDC} is the conditional stability constant and $[PDC']$ is the concentration of PDC not complexed to zinc. Normally, the concentration of PDC added to solution is much greater and than that of zinc, hence the total PDC concentration (C_{PDC}) added can be used instead of $[PDC']$. In our titrations we assumed that zinc formed a one to one stoichiometric complex with PDC, however assuming a 2:1 (PDC:Zn) complex would make no difference to the results because the α_{ZnPDC} was calibrated at the same PDC concentration as used in this work. A value of 4.4 was used for log K'_{ZnPDC} for a salinity of 36 and a pH of 8.2 (Van den Berg, 1985; Donat and Bruland, 1990). Finally, once C_{L} and K'_{ZnL} have been determined the concentration of Zn^{2+} in solution was calculated by solving the following quadratic equation

$$[\mathrm{Zn}^{2+}]^2\alpha_{\mathrm{Zn}}K'_{\mathrm{ZnL}} + [\mathrm{Zn}^{2+}](K'_{\mathrm{ZnL}}C_{\mathrm{L}} - K'_{\mathrm{ZnL}}C_{\mathrm{Zn}} + \alpha_{\mathrm{Zn}}) - C_{\mathrm{Zn}} = 0 \quad (7)$$

where C_{Zn} is the total concentration of zinc in solution.

2. Experimental

2.1. Instrumentation and reagents

The voltammetric system consisted of an Autolab PSTAT10 (Eco Chemie, The Netherlands) and a static mercury drop electrode (Metrohm, 663VA) which were coupled to a Cardstar portable computer (486 PC). The reference electrode was double-junction, Ag/AgCl, KCl (3 M), saturated AgCl, with a salt bridge filled with 3 M KCl, and the counter

electrode was a glassy carbon rod. Water used to make up reagents was purified using a Millipore reverse-osmosis, ion-exchange water purification system (designated here as Milli-Q water). Acids and ammonia solutions were purified by sub-boiling evaporation in a quartz still (designated here as Q-acid or Q-NH_3).

All plastic-ware used in this study was rigorously acid-cleaned. Typically, 1-l and 500-ml sample bottles were soaked for 1 week in a 50% HCl (AR grade) solution, rinsed with Milli-Q water, and then soaked for a further week in $\sim$2 M HNO_3 (AR grade). Finally the bottles were rinsed with Milli-Q water, filled with 0.5% Q-HCl and then doubly bagged. Laboratory plastic-ware was acid-cleaned by heating for three days in $\sim$25% aqua regia followed by 1 week soaking in 0.5% Q-HNO_3. Plastic-ware was rinsed with Milli-Q water before transfer between acid baths and before usage.

A 1.5 M stock borate buffer solution was prepared by dissolving boric acid in a 0.4 M NaOH solution. The buffer solution was cleaned by passing it through a chelex-100 column followed by UV digestion. Additions of 50 μl of buffer to 10 ml of seawater gave a pH of 8.2 (NBS pH scale). A 0.013 M stock solution of APDC (Fisher Scientific) was prepared every 2–3 weeks in a $\sim$0.1% Q-NH_3 solution, which was stored in a 30-ml Teflon bottle. The APDC stock solution was used ''as is'' as no blank was detected ($<$0.05 nM). Zinc standards were prepared in 0.05% Q-HCl by serial dilution of a 1000 mg kg^{-1} standard (BDH Chemicals). A 1.5 M HEPPS (4-(2-hydroxyethyl)-piperazine-1-propane-sulfonic acid; Fluka) buffer was prepared in $\sim$1.2 M Q-NH_3; additions of 50 μl HEPPS buffer to 10-ml sample gave a pH of 8.1. The HEPPS buffer was used ''as is'' as no zinc blank was detected.

2.2. Total zinc determinations

Total zinc was determined by CSV after UV-digestion of samples in either silica tubes or in a Teflon sample cup for a period of 45 min. Silica tubes were conditioned before use with each sample to minimise the loss of zinc due to adsorption onto tube walls as irradiation was carried without sample acidification. Recovery tests with added zinc (1 nM) yielded values within 5% of the expected value. After UV digestion, 7.5 mM of buffer and 120 μM of APDC were added to the sample. The sample was then deaerated for 4 min. Voltammetric conditions were: deoxygenation time 4 min; adsorption potential $-$1.3 V; adsorption time 60 to 90 s from stirred solution; reoxidation potential $-$0.8 V for 10 s; and a potential scan using square-wave modulation (50 Hz) with a step potential of 2.5 mV. After replicate scans were obtained the procedure was repeated twice with 1 nM standard additions of zinc.

2.3. Zinc titrations

To a 142-ml aliquot of seawater 7.5 mM of borate buffer and 60 μM of APDC were added. Ten 10-ml aliquots were then pipetted into ten 15-ml Teflon vials (Cole-Palmer) which were spiked with an appropriate amount zinc. Before use the Teflon vials were acid-cleaned and then conditioned ($3\times$) to minimise the effects of Zn–PDC adsorption onto vial walls. Samples were equilibrated overnight at room temperature ($\sim$20°C) before the zinc–PDC complex was determined using CSV. Voltammetric parameters were: a purge time of 4 min; adsorption potential $-$0.3 V; adsorption time 3 min; 10 s quiescence time; CSV scan from $-$0.8 to 1.2 V using square-wave modulation (50 Hz), with a step potential of 2.5 mV.

2.4. Sample collection

Samples were collected from the NE Atlantic during a cruise (76/91) with the RRV *Challenger* in March 1991 at 48°17.8′N, 12°28.8′W (station 13, Fig. 1). Samples were collected in pre-cleaned 10-l Teflon-coated Go-Flo bottles (General Oceanics). Go-Flo bottles were attached to a modified CTD-rosette (Morley et al., 1988) and collected on the up-cast. Upon recovery the Go-Flo bottles were transferred into a ''clean container'' with filtered air where the seawater was pressure filtered (0.5 Bar) through 0.4 μm acid-cleaned polycarbonate membrane filters using an in-line Teflon filtration unit into sample bottles. The sample bottles were re-bagged and immediately frozen.

Transect samples from the sea surface (3 m) were collected during a cruise (March 1998) with the RV *Pelagia* (Netherlands) as part of the MERLIM pro-

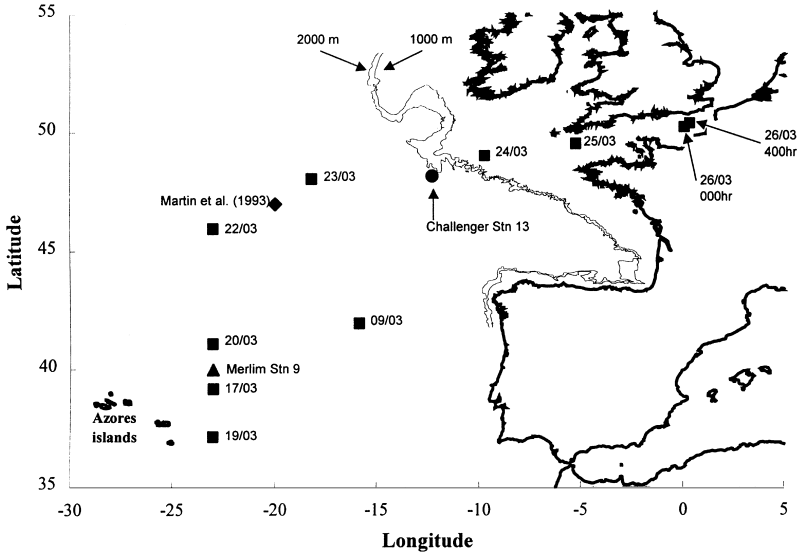

Fig. 1. Map of the Northeastern Atlantic Ocean showing positions of the sampling stations, transect samples and the JGOFS station of Martin et al. (1993).

ject of the EU. Using a peristaltic pump (flow rate 4 l min^{-1}) water was pumped via Teflon hose attached to a plastic ''fish'' positioned next to the research vessel. Seawater was continuously flushed through the tubing, and subsamples were filtered through a 0.2 μm filter cartridge (Sartobran). Depth samples during the MERLIM cruise were collected using 10-l Go-Flo bottles attached to a Kevlar line. Upon recovery Go-Flo bottles were transferred into a class 100 clean-container where sample bottles were filled, re-bagged and frozen (unfiltered).

A second set of samples (all filtered) was collected from the transect and water column of the MERLIM cruise and stored after acidification to provide a more comprehensive data set on the zinc distribution in this area; these can be used as a check

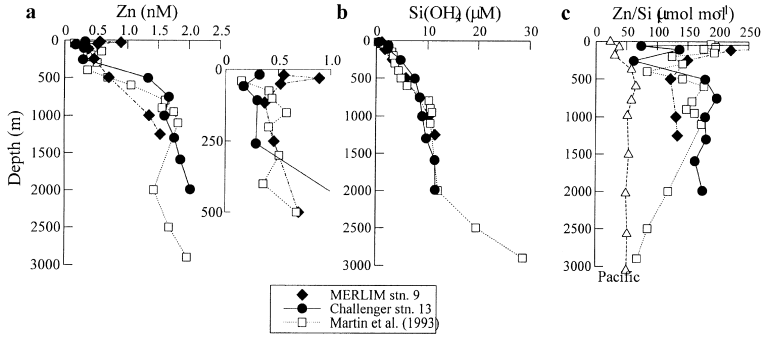

Fig. 2. Vertical profiles of (a) zinc, (b) silicate and (c) Zn/Si for stations 9, 13 and JGOFS (Martin et al., 1993). Inserted is a scale expansion of the zinc data for the upper water column. Pacific Zn/Si data was taken from Bruland (1980) for station 17 located at 32°41′N, 144°59′W.

on possible zinc losses due to the frozen sample storage (Nolting et al., submitted).

3. Results

3.1. Total zinc concentrations

Zinc results for the northeastern region of the Atlantic Ocean are presented in Fig. 2a, Fig. 3 and Tables 1–3. The two profiles determined in this study appear to be oceanographically consistent as they show the expected nutrient-like profile for zinc (Boyle et al., 1977). The samples had been stored frozen to preserve the speciation; possible zinc losses due to adsorption or precipitation were minimised by the use of large (0.5 and 1 L) samples and careful, slow (over a day), thawing followed by swirling of the bottles. Measurements of zinc in acidified samples from the MERLIM cruise (Nolting et al., submitted) appear to indicate higher zinc concentrations; however, this could be due to the use of different analytical methods including blank correction, and it is not yet substantiated whether any zinc losses occurred due to the frozen storage. Previous work has indicated no significant losses of zinc or ligands from sea water stored at natural pH at room temperature over periods of days to weeks (Bruland, 1989)

Table 1
Zinc, silicate, ligand, log K'_{ZnL} and Zn^{2+} data for the Challenger and MERLIM cruises (water column profiles only)

Depth (m)	Zn (nM)	Si (μM)	Ligand (nM)	log K'_{ZnL}	Zn^{2+} (pM)
Challenger station 13					
Position 48°17.8′N, 12°28.8′W					
18	0.32	0.5	1.79	10.3	9.8
58	0.17	2.3	1.13	10.4	7.0
107	0.3	2.2	0.68	10.6	17
258	0.29	4.7	0.91	10.1	27
506[a]	1.34	7.5			
754	1.68	8.5	2.04	10.4	97
1003	1.61	9.0	1.66	10.2	173
1299	1.77	9.8	1.53	10.1	285
1592[a]	1.87	11.5			
1991	2.03	11.6	2.44	10.2	154
MERLIM station 9					
Position 40°00′N, 23°00′W					
20	0.55[b]	1.06			
30	0.89[b]?	1.05			
50	0.52[b]	1.12			
115	0.37[b]	1.68			
250	0.46[b]	3.08			
500	0.70[b]	5.74			
1000	1.36[b]	10.36			
1250	1.54[b]	11.51			

? = Questionable data point.
[a]No ligand and log K'_{ZnL} data was obtained for these depths due to problems during analysis.
[b]Zinc determinations were on unfiltered samples.

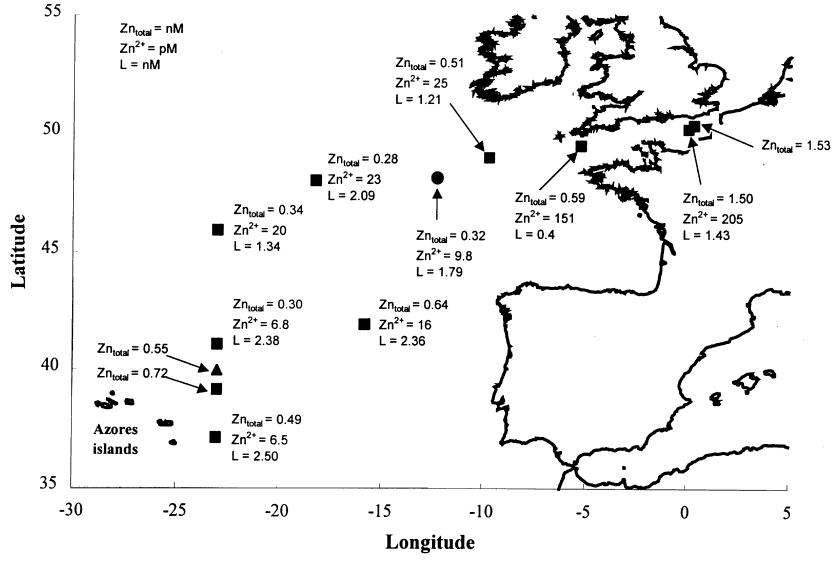

Fig. 3. Surface concentrations of total zinc, zinc-complexing ligand and free Zn^{2+} for each sampling site.

Table 2
Zinc, ligand and log K'_{ZnL} data for the transect samples collected on the MERLIM cruise from a depth of 3 m

Date and time sampled	Position	Zn (nM)	Ligand (nM)	log K'_{ZnL}	Zn^{2+} (pM)
09/3/98 (2400 h)	41°9′N, 15°83′W	0.64	2.36	10.3	16
17/3/98 (2000 h)	39°2′N, 22°99′W	0.72	–	–	
19/3/98 (2100 h)	37°1′N, 23°00′W	0.49	2.50	10.6	6.5
20/3/98 (2000 h)	37°1′N, 23°00′W	0.30	2.38	10.3	6.8
22/3/98 (0400 h)	46°0′N, 23°00′W	0.34	1.32	10.2	20
23/3/98 (0800 h)	48°1′N, 18°21′W	0.28	1.09	10.4	13
24/3/98 (1600 h)	49°0′N, 09°72′W	0.51	1.21	10.4	25
25/3/98 (0800 h)	49°5′N, 05°25′W	0.59	0.40	10.2	151
26/3/98 (0000 h)	50°2′N, 00°05′E	1.50	1.43	10.2	205
26/3/98 (0400 h)	50°4′N, 00°32′E	1.53	–	–	

indicating that such losses due to bottle adsorption are minor, possibly due to the organic complexation. We would have expected such losses to result in variable data, or disagreement with the known zinc distribution in the Atlantic (Martin et al., 1993). Silicate data from the frozen samples indicate no loss of that element either (see Station 13 below).

The zinc concentrations are in good agreement with previous zinc profiles that were collected in this region (Martin et al., 1993). Average surface water concentrations were 0.3 nM (Challenger station 13) and 0.5 nM (MERLIM station 9) compared to 0.4 nM at the JGOFS 47°N, 20°W station (Martin et al., 1993). Below 250 m zinc concentrations increased to 1.5 nM (MERLIM station 9) and 2 nM (Challenger station 13) compared to 1.8 nM at 1100 m at the JGOFS 47°N, 20°W station (Martin et al., 1993).

Each profile is characterised by zinc depletion in surface waters and with an increase in zinc concentration with depth, much like the silicate profiles collected at the same stations (Fig. 2b). Zinc and silicate concentrations in the Atlantic profiles, however, are not as closely correlated as they are in the Pacific. A plot of the ratio of zinc/silicate (Zn/Si) vs. depth shows a distinct maximum at about 1000 m (Fig. 2c). Mediterranean outflow water is known to contain elevated levels of zinc and other metals and is typically found at depths between 1000 and 1200

Table 3
Duplicate analysis of samples from the Challenger profile and transect samples collected on the MERLIM cruise. Also presented are the effects the borate and HEPPS buffer have on determining the concentration of the zinc-complexing ligand

Challenger station 13	Position	Depth (m)	Zn (nM)	Ligand (nM)	log K'_{ZnL}
Bottle no. 1		18	0.32	1.79	10.3
Bottle no. 2		18	1.56[a]	1.63	10.3
Bottle no. 1		58	0.17	1.11	10.4
Bottle no. 2		58	0.13	1.55	10.4
Bottle no. 2		58	0.13	1.16	10.3
Transect					
23/3/98 (0800 h)	48 1N, 18 21W	3	0.28	1.09	10.4
23/3/98 (0800 h)	48 1N, 18 21W	3	0.28	1.09	10.2
Buffers					
09/3/98 Borate buffer	41 9N, 15 83W	3	0.64	2.36	10.3
09/3/98 HEPPS buffer	41 9N, 15 83W	3	0.64	1.31	10.3
09/3/98 Borate buffer	37 1N, 23 00W	3	0.30	2.38	10.3
20/3/98 HEPPS buffer	37 1N, 23 00W	3	0.30	1.26	10.2

[a] Sample bottle was contaminated for zinc.

m in this region of the Atlantic Ocean (Sherrell and Boyle, 1988; Van Geen et al., 1991; Measures et al., 1995; Yeats et al., 1995). Perhaps the maximum in the Zn/Si data is due to Mediterranean outflow water, with higher zinc concentrations pervading into this region of the North Atlantic.

Zinc concentrations for the surface transect samples were low in open-ocean waters with a concentration of about 0.3 nM. Zinc levels increased landward to reach a value of 1.53 nM in the Channel (Fig. 3, Table 1). Higher zinc concentrations also occurred in samples collected near the Azores islands.

3.2. Zinc speciation data

A typical zinc titration curve is presented in Fig. 4a,b. The clear curvature in the titration data at low zinc concentrations indicates the presence of a zinc binding ligand. As the zinc concentration was increased the electrode response became linear indicating that effectively all of the zinc complexing ligand had been titrated with zinc. Plots of $[Zn_{labile}]/[ZnL]$ vs. $[Zn_{labile}]$ were linear indicating that a simple one-ligand one-metal model could be used to describe the linearised data (Ruzic, 1982; Van den Berg, 1982). For example, the data from Fig. 4a (a transect sample) was transformed using Eq. (3) to yield a ligand concentration of 2.36 ± 0.05 nM with a conditional stability constant ($\log K'_{ZnL}$) of 10.3 ± 0.2 (Fig. 4b). The one-ligand one-metal model was applied to all sample titrations carried out in this study.

A number of replicate titrations were carried out to determine method reproducibility. Replicate titrations were generally in good agreement with an average variation in the ligand concentration of ~ 0.1 nM and in the conditional stability constant (log value) of ~ 0.1 (Table 3).

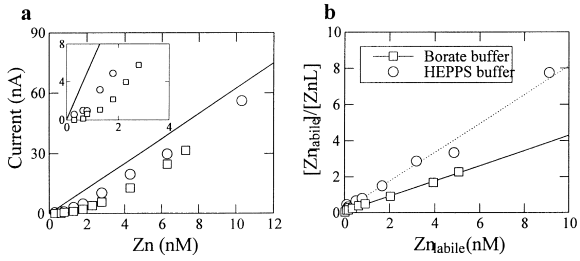

Fig. 5. Titration of transect sample 20/03 with the borate buffer and the HEPPS buffer. (a) Peak current vs. added zinc concentration for each buffer. The inserted figure is a scale expansion of the lower current values (note that the borate data has been normalised to HEPPS data to aid comparison). (b) Linearisation of the data from which a ligand concentration of 2.38 nM and a log $K'_{ZnL} = 10.3$ were calculated for the titration with the borate buffer. The linearised data for the titration with the HEPPS buffer yielded a ligand concentration of 1.15 nM and a log K'_{ZnL} of 10.5.

3.3. Effects of different pH buffers

The HEPPS pH buffer was initially used for complexing ligand titrations. However, curvature of the titrations was found to be weak indicating that either the ZnL complex was weak or the competition by the ZnPDC complex and side-reactions with the buffer were strong. Comparative titrations using the borate pH buffer gave more pronounced curvature in the titrations (using the same APDC concentration) indicating that zinc complexation by HEPPS was significant ($\alpha_{ZnHEPPS} > \alpha_{Zn} \sim 2$). Comparative titrations of the same sample with the two different buffers are presented in Fig. 5. A ligand concentration of 2.38 nM, log K'_{ZnL} of 10.3, was obtained with the borate pH buffer, whereas with the HEPPS

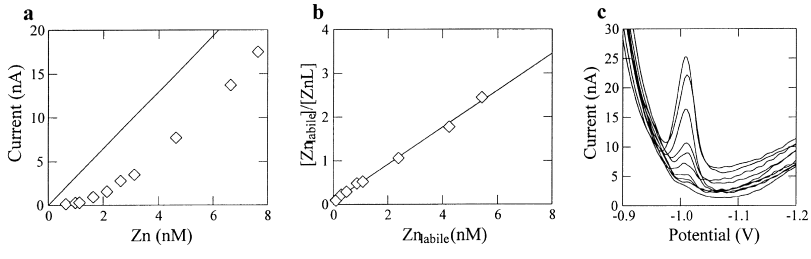

Fig. 4. Zinc titration data for transect sample 09/03. (a) Peak current vs. added zinc concentration. (b) Linearisation of the data from which a ligand concentration of 2.36 nM and a log $K'_{ZnL} = 10.3$ were calculated. (c) Voltammetric scans for zinc additions.

buffer a ligand concentration of 1.26 nM, log K'_{ZnL} of 10.2, was obtained. A similar discrepancy of ~ 1 nM in the ligand concentration was obtained for a second sample using the two different buffers (Table 3). It should be noted that the differences were not due to a higher zinc blank in the HEPPS buffer compared to the borate buffer as the zinc concentration in both buffers was less than 0.05 nM.

To avoid the interference by side-reactions of zinc with HEPPS all complexing ligand titrations were carried out using the borate pH buffer, which has a very low complex stability with zinc (Van den Berg, 1984): at pH 8.2, for 0.0075 M borate, the $\alpha_{ZnB(OH)4} = 4 \times 10^{-4}$, much less than the complexation of Zn^{2+} by other anions in seawater ($\alpha_{Zn} = 2.1$).

3.4. Ligand concentrations, stability constants and zinc speciation

Ligand concentrations, conditional stability constants for the zinc–ligand complex and estimates of Zn^{2+} concentrations for the water column at station 13 and the Atlantic surface transect are presented in Figs. 3 and 6 and Tables 1 and 2. The ligand concentration in deep waters was fairly constant at a value of about 1.7 nM which was approximately equal to the zinc concentration. In surface waters, i.e., samples collected at depths less than 250 m, ligand concentrations varied between 0.4 and 2.50 nM but were always in excess of the zinc concentrations (Fig. 6). The conditional stability constants for the Zn–ligand complex changed little with sampling depth or location, remained fairly constant down the water column and for the surface transect with log K'_{ZnL} values ranging between 10.1 and 10.5 (log K'_{ZnL}) (Fig. 3; Table 1).

Zn^{2+} concentrations shown in Figs. 3 and 6 were calculated by solving Eq. (7). Surface Zn^{2+} concentrations ranged between 6.5 pM for the open-ocean samples to 205 pM for samples collected in the Channel (Fig. 3). For the profile at station 13 the Zn^{2+} concentration ranged from 7 pM in surface waters to at maximum of 285 pM in deeper waters. For open-ocean surface waters between 96 and 99% of zinc is complexed to an organic ligand.

4. Discussion

At present, little is known about zinc speciation in open-ocean regimes due to the difficulty in collecting uncontaminated samples for determining total zinc and zinc-complexing ligand concentrations. The results presented here for zinc and the zinc-binding ligand appear to be oceanographically consistent suggesting that the samples were uncontaminated with zinc.

The concentrations of the zinc-complexing ligands in the northeastern Atlantic are similar to those in the North Pacific (Bruland, 1989; Donat and Bruland, 1990). The Atlantic K'_{ZnL} values (average log $K'_{ZnL} = 10.3$) are similar to those determined by CSV in the Pacific (log $K'_{ZnL} = 10.3$; Donat and Bruland, 1990) but smaller than those determined by anodic stripping voltammetry (ASV) for the Pacific (average log $K'_{ZnL} = 11.0$) (Bruland, 1989). Appar-

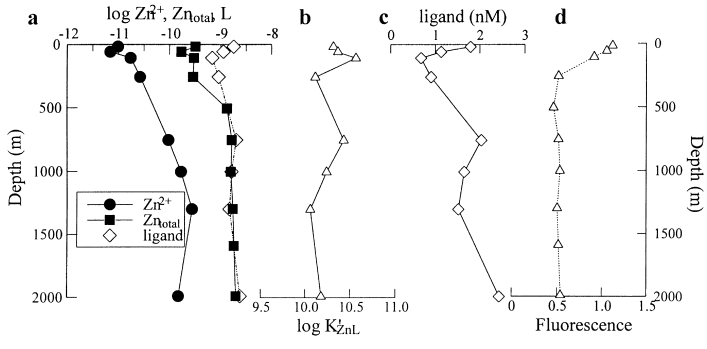

Fig. 6. (a) Vertical profiles of Zn^{2+}, dissolved zinc and the natural zinc-complexing ligand (log scales). (b) Vertical distribution of log K'_{ZnL}. (c) Vertical distribution of the zinc-complexing ligand. (d) Variations in fluorescence with depth.

ently, K'_{ZnL} values obtained using ASV are systematically higher than those obtained by CSV in otherwise similar conditions (Donat and Bruland, 1990).

4.1. Systematic difference between ASV and CSV

The discrepancy between the K'_{ZnL} values obtained by ASV and CSV causes a systematic difference in the estimated seawater Zn^{2+} concentrations. For example, for Pacific surface seawater with a zinc concentration 0.1 nM and a zinc-complexing ligand concentration of 1.5 nM, the Zn^{2+} concentration would be either 0.7 or 3.3 pM at log K'_{ZnL} values of 11.0 or 10.3, respectively. Choosing one or the other would make a major difference in the prediction whether zinc may limit oceanic primary productivity or not: at a Zn^{2+} concentration buffered at 0.7 pM the coastal marine diatom *Thalassiosira pseudonana* is zinc-limited in culture, whilst at 3.3 pM of Zn^{2+} the diatom is able to grow at near maximum rates (Sunda and Huntsman, 1992, 1996). Suggestions that certain parts of the world ocean might be zinc-limited (Bruland, 1989; Morel et al., 1994) are based on the ASV speciation data which produced higher K'_{ZnL} values compared to CSV. Clearly the dependence of log K'_{ZnL} with speciation technique needs to be clarified.

Comparative measurements have shown that CSV and ASV give the same ligand concentrations for zinc but with K'_{ZnL} values differing by about one decade (Donat and Bruland, 1990) suggesting a problem with either or both methods in that aspect only. The systematic difference between the two methods can be due either to an overestimation of reactive zinc by CSV, or an underestimation of labile zinc by ASV, or to a combination of these. Donat and Bruland (1990) have suggested that the lower K'_{ZnL} values found with CSV could be due to a slight contribution to the zinc reduction current from electroreducible complexes; there are two ways in which this could hypothetically happen: (a) ZnL could behave as an adsorptive, electroactive Zn species, adsorbing on the mercury drop and producing a peak indiscernible from that of ZnPDC (with a very similar reduction potential); and (b) ZnL could dissociate in the diffusion layer during the ZnPDC deposition step to compensate for the adsorptive loss of ZnPDC.

We investigated the first possibility (a) by CSV measurements without the added competing ligand, with and without added zinc to saturate the natural ligand with zinc (Fig. 7). No zinc reduction peaks were apparent without the presence of PDC indicating that this effect was insignificant. The second possibility (b) is unlikely as about 50% of the zinc occurs as ZnPDC and its depletion during deposition is only slight. Furthermore, the ZnPDC complex stability has been calibrated against EDTA so any such effects (if significant) would presumably be automatically accounted for.

Another cause could be the formation of mixed complexes (LZnPDC) between Zn^{2+}, PDC, and L. This is possible only if such a complex is more stable than ZnL, ZnPDC or $Zn(PDC)_2$. It is not likely that such a species would adsorb and contribute to the peak because adsorptive species require a special, flat, structure to hold the Zn^{2+} at the electrode surface; further coincidence would be required to give a peak indiscernible from that for ZnPDC. More likely this hypothetical mixed species would act as a competing species, thus masking ZnL or as a second species (not likely in view of the agreement between ASV and CSV for ligand concentrations). No evidence for such a hypothetical species exists but this could be investigated using suitable model compounds for the unknown ligands of type L.

Hypothetical problems with ASV are several, depending on the plating and scanning stages of the measurement and are as follows.

(a) Complex dissociation in the diffusion layer because of depletion of inorganic zinc (of which there is very little) during the deposition step: this

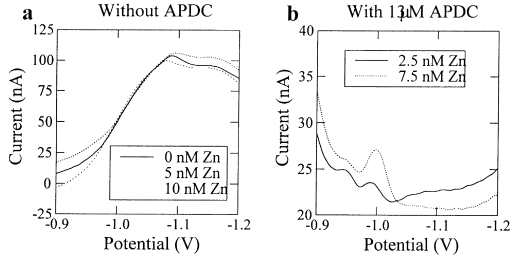

Fig. 7. Experiment to indicate lack of adsorption of natural zinc species on the electrode: voltammetric scans of transect sample 20/03 with 2.38 nM of ligand (a) without added APDC; (b) with APDC (13 μM).

would tend to lead to an overestimation of the labile metal concentration, as demonstrated for natural complexes with copper (Van den Berg, 1992).

(b) Direct reduction of ZnL during plating which is at an overpotential: it is likely that this would lead to an overestimation of the labile metal concentration.

(c) During the ASV scan, using a square-wave or pulsed scan modulation, the zinc is oxidised and reduced forth and back into and out of the diffusion layer where there is an excess of depleted natural ligands especially at low zinc concentrations: a possibility is that these ligands suppress the response of zinc at low zinc concentrations causing the very low peak heights characteristic of the ASV monitored titrations with zinc (Bruland, 1989): this would tend to underestimate the labile zinc concentration.

Direct ZnL reduction in ASV (b) was minimised by using a relatively small overpotential, and a rotating disk electrode was used to minimise the shifts in the equilibria (a) (Bruland, 1989). On the other hand, possible effects during the ASV scan (c) were not minimised and could be an explanation for the systematic difference. One way to test whether ASV underestimates the labile zinc concentration is to use medium exchange prior to the scan, either by physically changing the electrolyte solution or by adding a competing ligand to convert the re-oxidising zinc into an electrochemically reversible complex; this has been done successfully for copper (Scarano et al., 1992).

4.2. Buffer effect

Comparison of the ligand concentration detected with the HEPPS and borate pH buffers (Table 3) show that lower concentrations were obtained with the HEPPS buffer. This suggests that HEPPS is also complexing zinc in solution thus leading to an uncompensated overestimation of [Zn′], and therefore an underestimation of natural ligand concentrations. This is potentially an important finding as the HEPPS buffer has been used before in studies to determine the speciation of zinc, iron, nickel and copper in seawater (e.g., Donat et al., 1994; Rue and Bruland, 1995; Aldrich and Van den Berg, 1998). If HEPPS also significantly binds other metals then the concentration or complex stability of these other metal complexing ligands may also be underestimated.

4.3. Zn limitation?

Zn^{2+} rather than organically complexed zinc is thought to be the major zinc species taken up by phytoplankton (Anderson et al., 1978; Sunda and Huntsman, 1992). Zn^{2+} concentrations for the open Pacific Ocean range between about 2 and 14 pM (CSV data from Donat and Bruland, 1990) and between 6.8 and 20 pM for the open Atlantic Ocean (Fig. 3). At such Zn^{2+} concentrations zinc limitation of phytoplankton is possible for coastal species but is unlikely for open-ocean species (Brand et al., 1983; Sunda and Huntsman, 1992). Natural community incubation experiments have thus far not shown any evidence for zinc limitation in open-ocean waters (Coale et al., 1996).

The complexation of zinc by natural ligands in seawater is curious and raises some interesting questions. For example, what is the composition and source of these ligands which give greater complex stability with zinc than EDTA in seawater; it is not known whether they are produced by phytoplankton, or whether they are zinc specific, and how these ligands are distributed spatially and temporally. It has been hypothesised that these zinc-complexing ligands are produced by certain phytoplankton to lower Zn^{2+} concentrations at the expense of other phytoplankton (Bruland, 1989). The distribution of the ligand in the Atlantic water column (Fig. 6c) shows higher concentrations in surface waters which decrease to a minimum at 107 m followed by an increase with depth to 750 m. Below 750 m, the ligand concentration is relatively constant. If the ligand is being produced by phytoplankton, a maximum in the upper water column would be expected, not a minimum unless the water had recently become subducted. The variation across the productive upper water column therefore suggests that microorganisms in general may be responsible, or a distribution of excreted ligands is overshadowed by that from lysed cells.

If these zinc-complexing molecules were being directly produced by phytoplankton, one would expect to see a correlation between the two. No such correlation between fluorescence, a proxy for phytoplankton concentration, and natural concentrations is apparent from these data (Fig. 6c,d). One of the shortcomings with ligand production by phytoplank-

ton is that the energy required to produce such a ligand would lower the return each individual cell would obtain from its production (Bruland, 1989). Nevertheless algae are known to produce large quantities of ligands in response to toxic Cu^{2+} concentrations and for iron when cells are iron-limited (Moffett and Brand, 1996; Rue and Bruland, 1997; Boye and Van den Berg, 1999; Leal and Van den Berg, 1999).

One possibility is that the zinc binding ligand is a degradation product or enzyme from dead and decaying organisms sinking out of the euphotic zone. The relatively constant spatial temporal distribution of this ligand in deep waters of both the North Pacific and the Atlantic would be compatible with a ligand that is either a stable molecule or is produced in situ by bacteria or decaying phytoplankton (Fig. 6c).

Zinc ions are known to stabilise proteins structurally by forming a ''zinc finger'' which allows polypeptide loops to fold around the zinc ion thus holding the polypeptide molecules in a rigid domain (Klug and Schwabe, 1995; Barnes et al., 1998). These polypeptide molecules, rich in sulphur and nitrogen, are known to form very strong complexes with zinc, stronger than EDTA (Barnes et al., 1998). Perhaps the zinc-binding ligands found in ocean waters are polypeptide molecules produced during the decay of phytoplankton.

5. Conclusions

Zinc concentration profiles produced in this study are consistent with previous ones measured for the northeastern Atlantic Ocean. For open-ocean surface waters zinc speciation is dominated by zinc complexation to an unidentified organic ligand, and less than 4% of zinc occurs as inorganic species. For transect samples ligand concentrations ranged from 0.40 to 2.50 nM and with log K'_{ZnL} values ranging between 10.2 and 10.6. In deeper waters zinc speciation was still dominated by complexation to the zinc binding ligand. Ligand concentrations range between 0.68 nM at 107 m and 2.44 nM at 1991 m and log K'_{ZnL} values ranged between 10.6 and 10.1. The ligand concentrations and the K'_{ZnL} constants measured here are comparable to those measured for zinc by the same method in the North Pacific Ocean.

Discrepancies were observed between the use of HEPPS and borate used to buffer samples during titrations. Replicate titrations with each buffer suggest that HEPPS weakly binds zinc in solution thereby affecting the analytically determined concentration of the oceanic zinc-complexing ligand. Differences were also observed between the K'_{ZnL} values produced in this study with CSV and data produced by Bruland (1989) using ASV. These differences in K'_{ZnL} can seriously affect the estimation of free Zn^{2+} concentrations in seawater and therefore estimates of zinc-limitation of phytoplankton.

Acknowledgements

This research was funded by grants from NERC (PRIME, GST/021058) and the EU (MERLIM, MAS3-CT95-0005). We thank Jeroen de Jong and Rob Nolting (NIOZ, Netherlands) for assistance with sample collection from station 9 on the MERLIM cruise.

References

Aldrich, A.P., Van den Berg, C.M.G., 1998. Determination of iron and its redox speciation in seawater using catalytic cathodic stripping voltammetry. Electroanalysis 10, 369–373.

Anderson, M.A., Morel, F.M.M., Guillard, R.R.L., 1978. Growth limitation of a coastal diatom by low zinc ion activity. Nature 276, 70–71.

Barnes, M.H., Leo, C.J., Brown, N.C., 1998. DNA polymerase III of Gram-positive eubacteria is a zinc metalloprotein conserving an essential finger-like domain. Biochemistry 37, 15254–15260.

Boye, M., Van den Berg, C.M.G., 1999. Iron availability and the release of iron complexing ligands by *Emiliania huxleyi*. Mar. Chem., submitted.

Boyle, E.A., Sclater, F.R., Edmond, J.M., 1977. The distribution of dissolved copper in the Pacific. Earth Planet. Sci. Lett. 37, 38–54.

Brand, L.E., Sunda, W.G., Guillard, R.R.L., 1983. Limitation of marine-phytoplankton reproductive rates by zinc, manganese, and iron. Limnol. Oceanogr. 28, 1182–1198.

Bruland, K.W., 1980. Oceanographic distributions of cadmium, zinc, nickel and copper in the North Pacific. Earth Planet. Sci. Lett. 47, 176–198.

Bruland, K.W., 1989. Complexation of zinc by natural organic-ligands in the central North Pacific. Limnol. Oceanogr. 34, 269–285.

Bruland, K.W., Franks, R.P., 1983. Mn, Ni, Cu, Zn and Cd in the Western North Atlantic. In: Wong, C.S., Boyle, E.A., Bruland,

K.W., Burton, J.D., Goldberg, E.D. (Eds.), Trace Metals in Seawater. NATO Conference Series. Plenum, New York, pp. 395–414.

Bruland, K.W., Knauer, G.A., Martin, J.H., 1978. Zinc in northeast Pacific waters. Nature 271, 741–743.

Candelone, J.P., Hong, S.M., Pellone, C., Boutron, C.F., 1995. Post-industrial revolution changes in large-scale atmospheric-pollution of the northern-hemisphere by heavy-metals as documented in central Greenland snow and ice. J. Geophys. Res. [Atmos.] 100, 16605–16616.

Coale, K.H., Johnson, K.S., Fitzwater, S.E., Gordon, R.M., Tanner, S., Chavez, F.P., Ferioli, L., Sakamoto, C., Rogers, P., Millero, F., Steinberg, P., Nightingale, P., Cooper, D., Cochlan, W.P., Landry, M.R., Constantinou, J., Rollwagen, G., Trasvina, A., Kudela, R., 1996. A massive phytoplankton bloom induced by an ecosystem-scale iron fertilization experiment in the equatorial Pacific Ocean. Nature 383, 495–501.

Donat, J.R., Bruland, K.W., 1990. A comparison of 2 voltammetric techniques for determining zinc speciation in northeast Pacific-Ocean waters. Mar. Chem. 28, 301–323.

Donat, J.R., Lao, K.A., Bruland, K.W., 1994. Speciation of dissolved copper and nickel in south San-Francisco Bay — a multimethod approach. Anal. Chim. Acta 284, 547–571.

Duce, R.A., Liss, P.S., Merrill, J.T., Atlas, E.L., Buat-Menard, P., Hicks, B.B., Miller, J.M., Prospero, J.M., Arimoto, R., Church, T.M., Ellis, W., Galloway, J.N., Hansen, L., Jickells, T.D., Knap, A.H., Reinhardt, K.H., Schneider, B., Soudine, A., Tokos, J.J., Tsunogai, S., Wollast, R., Zhous, M., 1991. The atmospheric input of trace species to the World Ocean. Global Biogchem. Cycles 5, 193–259.

Hong, S.M., Candelone, J.P., Boutron, C.F., 1997. Changes in zinc and cadmium concentrations in Greenland ice during the past 7760 years. Atmos. Environ. 31, 2235–2242.

Klug, A., Schwabe, J.W.R., 1995. Protein motifs: 5. Zinc fingers. FASEB J. 1995, 597–604.

Leal, M.F.C., Van den Berg, C.M.G., 1999. Copper induced release of complexing ligands similar to thiols by Emiliania huxleyi in seawater cultures. Limnol. Oceanogr., in proof under revision.

Martin, J.H., Fitzwater, S.E., Gordon, R.M., Hunter, C.N., Tanner, S.J., 1993. Iron, primary production and carbon nitrogen flux studies during the JGOFS North Atlantic bloom experiment. Deep-Sea Res., Part II 40, 115–134.

Measures, C.I., Yeats, P.A., Schmidt, D., 1995. The hydrographic setting of the IOC base-line cruise to the eastern Atlantic 30°S to 35°N. Mar. Chem. 49, 243–252.

Moffett, J.W., Brand, L.E., 1996. Production of strong, extracellular Cu chelators by marine cyanobacteria in response to Cu stress. Limnol. Oceanogr. 41, 388–395.

Morel, F.M.M., Reinfelder, J.R., Roberts, S.B., Chamberlain, C.P., Lee, J.G., Yee, D., 1994. Zinc and carbon co-limitation of marine-phytoplankton. Nature 369, 740–742.

Morley, N.H., Fay, C.W., Statham, P.J., 1988. Design and use of a clean shipboard handling system for seawater samples. Adv. Underwater Technol. 16, 283–289.

Nolting, R.F., Heijne, M., De Jong, J.T.M., Timmermans, K.R., De Baar, H.J.W., submitted. Distribution and behaviour of Zn, Cu and Cd in the northeast Atlantic Ocean. Mar. Chem.

Nriagu, J.O., 1989. A global assessment of natural sources of atmospheric trace-metals. Nature 338, 47–49.

Rue, E.L., Bruland, K.W., 1995. Complexation of iron(III) by natural organic-ligands in the central north Pacific as determined by a new competitive ligand equilibration adsorptive cathodic stripping voltammetric method. Mar. Chem. 50, 117–138.

Rue, E.L., Bruland, K.W., 1997. The role of organic complexation on ambient iron chemistry in the equatorial Pacific Ocean and the response of a mesoscale iron addition experiment. Limnol. Oceanogr. 42, 901–910.

Ruzic, I., 1982. Theoretical aspects of the direct titration of natural-waters and its information yield for trace-metal speciation. Anal. Chim. Acta 140, 99–113.

Scarano, G., Bramanti, E., Zirino, A., 1992. Determination of copper complexation in sea water by a ligand competition technique with voltammetric measurement of the labile metal fraction. Anal. Chim. Acta 264, 153–162.

Sherrell, R.M., Boyle, E.A., 1988. Zinc, chromium, vanadium and iron in the Mediterranean Sea. Deep-Sea Res., Part A 35, 1319–1334.

Sunda, W.G., Huntsman, S.A., 1992. Feedback interactions between zinc and phytoplankton in seawater. Limnol. Oceanogr. 37, 25–40.

Sunda, W.G., Huntsman, S.A., 1996. Antagonisms between cadmium and zinc toxicity and manganese limitation in a coastal diatom. Limnol. Oceanogr. 41, 373–387.

Turner, D.R., Whitfield, M., Dickson, A.G., 1981. The equilibrium speciation of dissolved components in fresh-water and seawater at 25°C and 1 atm pressure. Geochim. Cosmochim. Acta 45, 855–881.

Vallee, B.L., Auld, D.S., 1990. Zinc coordination, function and structure of zinc enzymes and other proteins. Biochem. 29, 5647–5659.

Van den Berg, C.M.G., 1982. Determination of copper complexation with natural organic-ligands in sea-water by equilibration with MnO_2: 1. Theory. Mar. Chem. 11, 307–322.

Van den Berg, C.M.G., 1984. Speciation of boron with Cu^{2+}, Zn^{2+}, Cd^{2+} and Pb^{2+} in 0.7 M KNO_3 and in sea-water. Geochim. Cosmochim. Acta 48, 2613–2617.

Van den Berg, C.M.G., 1985. Determination of the zinc complexing capacity in seawater by cathodic stripping voltammetry of zinc APDC complex-ions. Mar. Chem. 16, 121–130.

Van den Berg, C.M.G., 1992. The effect of the deposition potential on the voltammetric determination of complexing ligand concentrations in sea-water. Analyst 117, 589–593.

Van Geen, A., Boyle, E.A., Moore, W.S., 1991. Trace-metal enrichments in waters of the gulf of Cadiz, Spain. Geochim. Cosmochim. Acta 55, 2173–2191.

Yeats, P.A., Westerlund, S., Flegal, A.R., 1995. Cadmium, copper and nickel distributions at 4 stations in the eastern central and south-Atlantic. Mar. Chem. 49, 283–293.