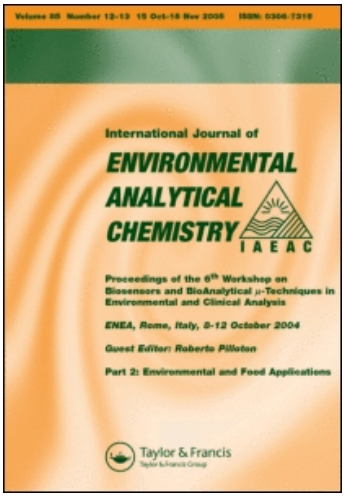

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Copper Complexation in the Surface Sea Water of Terra Nova Bay (ANTARCTICA)

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COPPER COMPLEXATION IN THE SURFACE SEAWATER OF TERRA NOVA BAY (ANTARCTICA)

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Speciation of copper in the surface seawater of Terra Nova Bay is studied by Differential Pulse Anodic Stripping Voltammetry using samples collected during the 1987–88, 1988–89 and 1989–90 Italian expeditions. Total copper concentration ranges between 0.5 and 4.8 nM and shows uniform spatial distribution without evident differences between the three campaigns. The mean value for the labile fraction is 4% of the total for the first two expeditions, while it is below the detection limit for the last one. Results show the presence of two classes of ligands, one stronger (mean concentration 1.5 nM) which shows considerable variability due to seasonal and spatial factors, and one weaker (mean concentration 31 nM, average conditional stability constant $2.8\times10^8\ M^{-1}$) which shows homogeneous distribution in the studied area.

KEY WORDS: Copper speciation, DPASV, seawater, Terra Nova Bay, Antarctica.

INTRODUCTION

In seawater the complexation of metals by organic ligands strongly affects their bioavailability and toxicity[1,2] and there is evidence that metal availability may be an important selective force acting on the population and communities of marine phytoplankton[1,3]. It has also been suggested that complexation of metals plays an important role in determining their reactivity and geochemical behaviour[4].

Copper has received considerable attention because of its requirement as a micronutrient and because of its toxicity[5–10]. Brand et al.[11] showed that natural levels of copper in recently upwelled seawater suppressed algae growth, and that the growth was restored when metal chelators or other micronutrient metals were added, suggesting that copper toxicity was due to the inhibition of enzymes which require other elements. Sunda and Guillard showed that the toxic effects of copper on phytoplankton are related to its ionic activity[12]. There is also evidence that copper may represent a biolimiting micronutrient for higher organisms and for phytoplankton. In particular, Manahan and Smith[13] found that an ionic copper activity of 10^{-16} M is the lower requirement limit for two freshwater algae, and Schenck[14] showed a limitation to the vitality of Ganyaulax Tamarensis when the cupric ionic activity was lower

than 10^{-13} M, while Anderson and Morel[1] showed that the same dinoflagellate reduces its motility by 100% when the ionic activity exceeds $10^{-9.7}$M.

Copper concentration in oceans appears to range between 0.5 and 6 nM, with an intermediate distribution between that of nutrient type elements and that of scavenged elements. There is higher surface concentration in the North Atlantic with respect to the North Pacific, and high nearshore concentrations normally associated with dissolved Mn maxima[15].

The adoption, in the past decade, of ultraclean sampling procedures and improvements in analytical methodologies have made it possible to deal with speciation problems. Several studies have been carried out using different techniques to investigate copper complexation in seawater; the results show that the fraction of copper complexed by organic ligands in surface seawater represents a large part of the total, normally higher than 99%[9,16–18]. The data is considerably variable. This may be due to differences of complexation between the areas studied or to differences in the labile fraction detection window of the techniques used. Speciation studies carried out with potentiometric measurements or with methodologies using internal calibration, such as bioassays[17] or adsorptive stripping voltammetry[18], directly determine ionic copper activity or concentration. Voltammetric methods detect a labile fraction composed of ionic metal, inorganic complexes and weakly bound organic complexes, such as Cu-Glycine or Cu-acetate. However, in seawater these weak organic ligands are present at very low concentrations and do not contribute substantially to copper complexation; we can therefore consider that the labile fraction is composed of free metal and its inorganic complexes.

Though more complex models have been proposed[19], copper speciation data generally fit models with two classes of ligands well, one stronger at low concentration and one weaker at higher concentration. Coale and Bruland[16], while studying the copper distribution along a profile in an oligothrophic area in the North Pacific, showed that the class of strong ligands exhibits a vertical distribution quite similar to the primary production, thus displaying a concentration maximum at a depth of about 50–70 m, after which a rapid decrease to zero is observed. Conversely, the second class of weak ligands is uniformly distributed along the vertical profile. These ligands suppressed the ionic copper activity to values of 10^{-14} M in surface and 10^{-11} M in water deeper than 200 m. Similar results are reported for the Atlantic Ocean[20].

Recently we have had the opportunity to investigate metal speciation in the seawater of the Antarctic coast (Ross Sea) during the 1987–88, 1988–89 and 1989–90 Italian expeditions. Pb, Cd and Cu complexation was studied titrating the sample with each one of the metals separately and detecting the labile fraction by Differential Pulse Anodic Stripping Voltammetry (DPASV). Previous results, obtained by studying the distribution and the speciation of Pb and Cd in the seawater of Terra Nova Bay, emphasize interesting relationships between speciation of these elements and biological activity[21,22]. In this paper, we report data relative to the study of Cu complexation in the surface seawater of Terra Nova Bay. Results obtained for samples collected during the three campaigns are compared in order to study the temporal variability of Cu concentration and its speciation.

EXPERIMENTAL

Sampling and pretreatment

Subsurface seawater samples were collected at a 0.5 m depth in Terra Nova Bay (Ross Sea, Antarctica) during three Italian expeditions (austral summer seasons 1987–88, 1988–89 and 1989–90). The sampling positions are indicated in Figure 1. Sampling was carried out using a Teflon pump operated from the prow of an inflatable dinghy moving upwind with respect to the research vessel. A 50 l polyethylene tank was used to collect samples for our investigations and for the activities of other scientists participating in the same expedition.

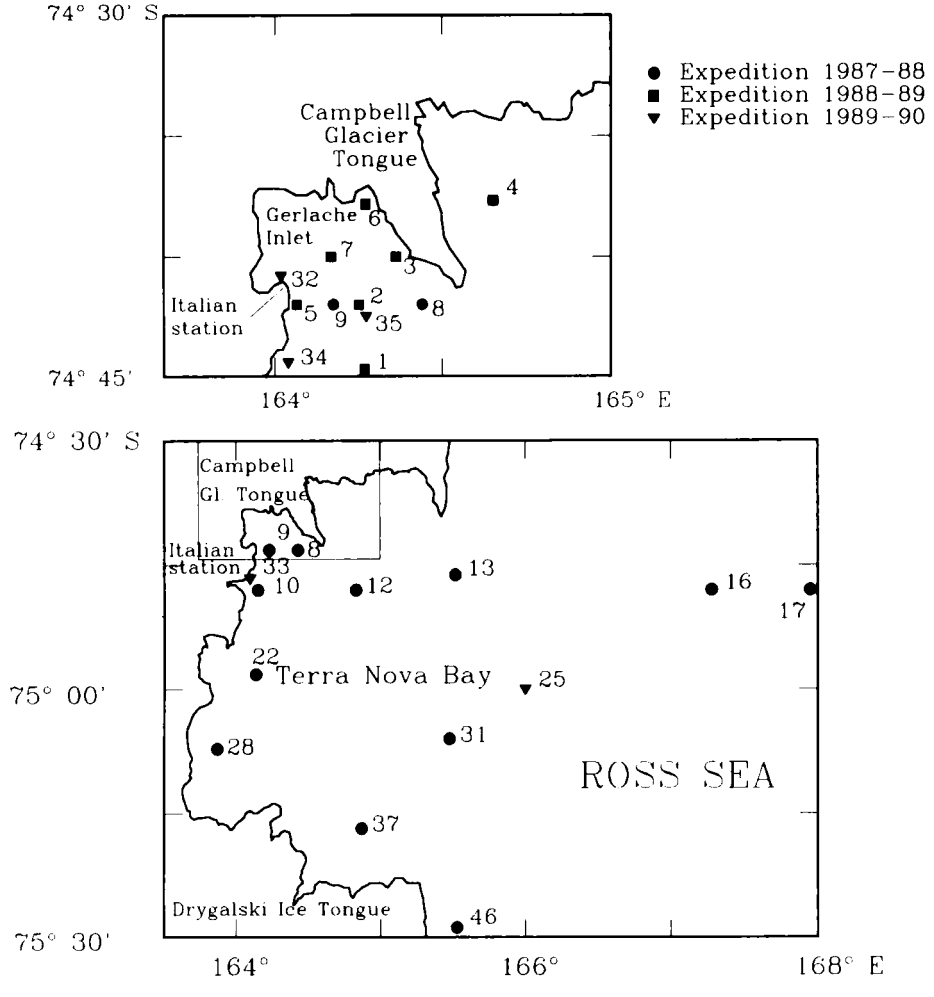

Figure 1 Sampling stations in Terra Nova Bay during the 1987–88, 1988–89 and 1989–90 expeditions.

Both the pump and the container were acid cleaned and seawater conditioned before use. Sample 25 of the 1989–90 expedition was gathered at a depth of 20 m with a Teflon coated GO-FLO sampler (General Oceanics).

Samples 5, 6 and 7 of the 1988–89 campaign were collected before and after pack melting to give relevance to the effects of ice melting on the metal distribution. Samples collected under ice were pumped to the surface using a Teflon pump according to a previously reported procedure[22].

Filtration of samples was performed by passing the seawater through a membrane filter sandwiched in a Teflon support (Sartorius SM16540). Acid cleaned and conditioned cellulose nitrate filters (142 mm diameter, 0.45 μm pore size) were used. To avoid risk of contamination, the first aliquote of filtrate (3–5 l) was discarded, the sample was subsequently collected in 1 or 2 l FEP bottles (acid cleaned and seawater conditioned) and stored frozen (−20°C).

Handling and treatment of samples were carried out in a clean chemical laboratory under Class 10 or Class 100 laminar flow hoods, both in Antarctica (on the ship and in the laboratory of the station) and in Italy.

Measurements on stored samples were carried out within six to eight months in the Venice laboratory. Aliquots of samples collected during the last expedition were also immediately analysed on site. Table 1 gives the results of the tests performed to verify the conservation procedure. It can be noted that no significant variation in the total concentration of copper occurred during storage, so we can be confident that the adopted procedure is adequate for preserving the integrity of samples over long periods of time.

Instrumentation

Differential Pulse Anodic Stripping Voltammetry measurements were carried out by a Multipolarograph Analyzer EG&G mod. 384 B equipped with an electrochemical cell EG&G Rotel 2, developed for ultra-trace metal determination. A rotating glassy carbon disk, on which a thin mercury film was deposited, was provided as a working electrode. A platinum auxiliary electrode and an Ag/AgCl, KCl saturated reference electrode, to which all the reported potentials are referred, were used. The electrochemical cell was devised for metal determination at ultralow levels and all the materials were cleaned and prepared according to the usual recommended procedure. The mercury film was prepared daily immediately prior to each analysis, according to the previously reported procedure[21].

Table 1 Comparison of results obtained immediately and after conservation (6–8 months) for samples collected during the 1989–90 expedition.

Station	*Total copper concentration, nM*	
	Before storage	*After storage*
25	2.3	1.9
33	2.1	2.0
34	2.5	2.3
35	1.8	1.4

To perform a blank control, after the film was prepared, a differential pulse voltammetric scan was carried out at a scan rate of 10 mV s^{-1}, pulse amplitude of 50 mV and pulse frequency 5 s^{-1}. If the voltammogram did not show peaks and the base line was satisfactory, the electrochemical cell was rinsed with an aliquot of sample previously purged with nitrogen to eliminate excess mercury (used for the preparation of the film), and finally the already deaerated sample was placed in the analytical position and the measurement was started.

Total concentration

Total concentration of dissolved copper (C_{Cu}) was determined in filtered samples. Aliquots were transferred into the Teflon cup of the electrochemical cell and acidified at pH 2 with HCl (Suprapur, Merck; Ultrapure, NIST) at least 48 hours prior to analysis in order to release metals complexed by organic ligands. The determinations were performed by DPASV, using the multiple standard additions method. Measurements were carried out by applying a deposition potential of –0.85 V (vs. Ag/AgCl, KCl sat) for 20 minutes (rotation speed 4000 rpm), a subsequent quiescent period of 30 seconds was allowed to pass while the electrode was kept at the same potential, and finally a differential pulse voltammetric scan was started (scan rate 10 mV s^{-1}, pulse amplitude 50 mV, pulse frequency 5 s^{-1}) in a positive direction until a final potential of –0.15 V was reached. The rotating electrode was held at –0.18 V for 5 minutes to remove the residual amalgamated metals completely before subsequent measurement was started.

The completeness of mineralization obtained by acid digestion was tested by comparing results with those obtained after UV irradiation of acidified samples for 6–24 hours (1.2 kW lamp Hanovia, Ace Glass Inc.). Results did not show significant differences in the copper concentration, thus confirming that acid treatment is sufficient for releasing copper from organic complexes (see Figure 2).

The blank for the digestion procedure was tested by the addition of HCl to a solution of KCl 0.03 M (100 µl of HCl 30% Suprapur Merck or 32% Ultrapure NIST to 50 ml of KCl solution), which was analyzed to detect the copper content before and after the addition of HCl. The difference in concentrations was lower than the detection limit for copper (0.02 nM), so the contribution from the blank was not considered to be influential on the determination of the total concentration.

Accuracy was periodically verified by analysing a standard seawater sample with a certified content of heavy metals (NASS-2 or NASS-3, National Research Council of Canada[23,24]). Table 2 reports the results of one of these tests. Measurements on samples were carried out only after results on reference samples came within the tolerance interval of the certified value for lead, cadmium and copper.

Repeatability of measurements of total copper concentration, computed from 5 repetitions carried out on sample 33, was 15% (as RSD) at the level of 2.1 nM.

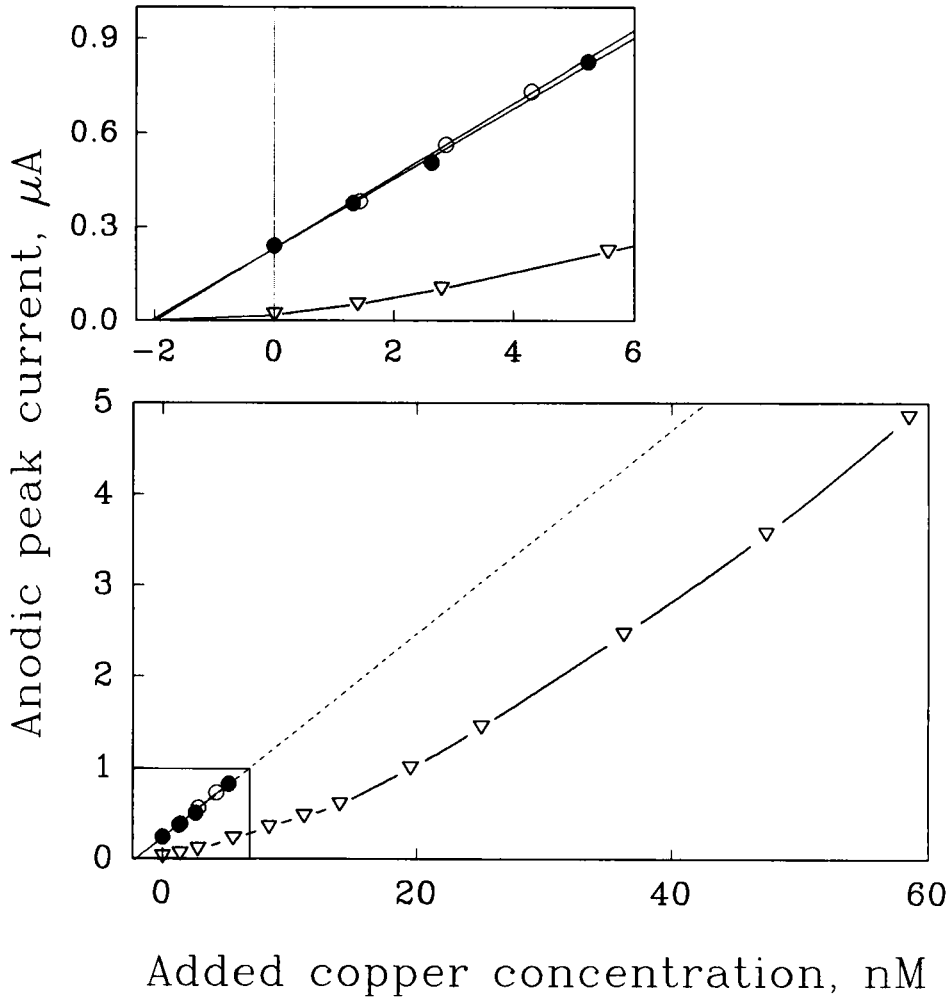

Figure 2 Titration curves obtained after different treatments of the sample: (▽) seawater at natural pH, (○) seawater acidified at pH 2 and UV irradiated (1.2 kW lamp, 10 h), (●) seawater acidified at pH 2. Experiment carried out on sample 31 (1987–88 expedition).

Speciation procedure

Theoretical considerations. The use of ASV to study speciation of metals in seawater has been developed by several researchers[25–29]. The approach is based on the ability of DPASV to differentiate two fractions of the metal: the first, ASV-labile, is composed of metal ion and metal complexes which are electroactive at the selected deposition potential (inorganic complexes and weak organic complexes); the second consists of inert forms of metals, i.e. strong organic complexes which are thermodynamically and kinetically stable.

Table 2 Typical concentration values (ng/l) obtained on the NASS-3 reference seawater material.

Element	*Found*		*Certified*
	Results	*Mean(±95%CI)*[a]	*Mean(±95%TI)*[b]
Cd	30,29,28,35,29,31	30(±3)	29(±4)
Pb	42,36,38,37,37,40	38(±2)	30(±6)
Cu	137,104,121,108, 119,111	117(±12)	109(±11)

([a]) In brackets 95% confidence interval.
([b]) In brackets 95% tolerance interval.

The procedure involves the titration of organic ligands present in the sample by copper, and the voltammetric measurement, initially and after each addition, of the oxidation peak current due to the labile fraction. This methodology derives from the assumption that copper forms non-electroactive complexes with strong ligands present in solution, following the reaction:

$$Cu^{2+}+L^{n-}=CuL^{2-n} \quad (1)$$

The stoichiometric stability constant for this reaction (the charges being omitted here and in the following for the sake of simplicity) is:

$$K=[CuL]/([Cu][L]) \quad (2)$$

where $K=K^*(\gamma_{Cu}\gamma_L/\gamma_{CuL})$, K^* being the thermodynamic stability constant and γ_i the activity coefficient of the species i. If species are involved in side reactions, other considerations must be made. In seawater, copper forms inorganic complexes[30] mainly with CO_3^{2-}, OH^- and Cl^- and the organic ligands may be coordinated by major cations such as Ca^{2+} and Mg^{2+} or other trace metals potentially competing with copper. The side reactions can change the concentration of the free metal ion and the free organic ligand considerably, and to account for their effect on the main equilibrium, the side-reaction coefficients α_{Cu}, α_L and α_{CuL}, as defined by Ringbom et al.[31,32], need to be introduced into the stability constant. In terms of side reaction coefficients, the concentrations of the free copper ion, the free ligand and the complex are $[Cu]=[Cu']/\alpha_{Cu}$, $[L]=[L']/\alpha_L$ and $[CuL]=[CuL']/\alpha_{CuL}$, respectively, where [Cu'] is the concentration of the dissolved copper present in all the inorganic forms, [L'] is the concentration of ligand not coordinated by copper and [CuL'] the copper complex present in all forms, even as mixed complexes (e.g. with OH^-, Cl^-). Considering the complex not involved in side reactions, i.e. [CuL']=[CuL], we can define the conditional stability constant as:

$$K'=K/(\alpha_{Cu}\alpha_L) \quad (3)$$

Carrying out titration, after each addition, the mass balance for copper is

$$Cu_t = [Cu'] + [CuL] = C_{Cu} + Cu_a \quad (4)$$

where Cu_t is the overall total concentration of copper, i.e. the sample total concentration

measured independently, C_{Cu}, plus the added amount, Cu_a. Considering the above-made assumption, and considering that the weak organic ligands do not contribute substantially to copper complexation, the conditional copper concentration, [Cu′], can be equated to the ASV-labile fraction and experimentally evaluated as

$$[Cu'] = i_p/S \tag{5}$$

where i_p is the peak current and S is the sensitivity (as the slope of the titration curve measured at high values of Cu_a, where ligands have been saturated). The concentration of complexed copper, [CuL], is calculated as the complement of [Cu′] to the total concentration

$$[CuL] = Cu_t - [Cu'] \tag{6}$$

If one ligand, or one class of ligands (i.e. ligands having approximately similar strength), is present and the stoichiometry of the reaction of complexation is 1:1, and if equilibrium is reached after each addition, the plot $[Cu']/(Cu_t-[Cu'])$ versus [Cu′] assumes a linear shape as defined by the equation[18,29,33]:

$$[Cu']/(Cu_t-[Cu']) = [Cu']/C_L + 1/(C_L K') \tag{7}$$

where C_L is the total ligand concentration.

If more ligands are present, the mass balance for copper will be

$$C_{Cu} = [Cu'] + \sum_{i=1}^{n} [CuL_i] \tag{8}$$

where the summation represents the sum of the concentrations of all the copper species derived from complexation by the different ligands present. In this case the transformed plot of titration data, as defined above, will assume a curved shape. From the expression of the i-th conditional stability constant (K'_i) relative to the reaction of complexation with the i-th ligand and the mass balance for ligand L_i we can obtain

$$[CuL_i] = \frac{[Cu']K_i'C_{L_i}}{1+K_i'[Cu']} \tag{9}$$

where C_{Li} is the total concentration of L_i. If n ligands are present, we can write

$$Cu_t - [Cu'] = \sum_{i=1}^{n} \left(\frac{C_{L_i}}{1+1/(K_i'[Cu'])} \right) \tag{10}$$

then

$$\frac{[Cu']}{Cu_t - [Cu']} = 1 / \sum_{i=1}^{n} \left(\frac{C_{L_i}}{[Cu'] + 1/k_i'} \right) \tag{11}$$

Studies of the speciation of copper fit well the model which considers two classes of ligands[16,18,34]; thus equation 11 becomes

$$\frac{[Cu']}{Cu_t - [Cu']} = 1/\left[\frac{C_{L1}}{([Cu'] + 1/K_1')} + \frac{C_{L2}}{([Cu'] + 1/K_2')}\right] \quad (12)$$

A nonlinear fitting of the experimental data to equation 12 can be applied to compute C_{L1}, C_{L2}, K'_1 and K'_2. In the present work the Marquart-Levenberg method was used[35].

Experimental procedure. Application of the ASV technique for studying the interaction of trace metals with organic ligands in natural waters has been the subject of some controversy regarding the possible reduction of organic complexed metals, thus determining overestimation of the labile fraction. However, accurate tests using model ligands were carried out to ensure rigorous definition of this technique in studying the speciation of zinc, lead and copper in seawater[16,36,37]. Reported results show that ligand concentration and conditional stability constants are in agreement with theoretical data. From this it can be clearly shown that results obtained by the application of ASV to speciation study are consistent with complexation of metals in natural waters.

The procedure adopted here was based on titration of the organic ligands present in the filtered sample by copper. DPASV measurements were initially carried out on the untreated sample and then after each addition, to detect the labile metal present. In each titration, 12 to 20 standard additions were made until a total concentration of about 90 nM was achieved. After each addition, the solution was equilibrated for 15 minutes before starting the measurement. The deposition potential, selected on the basis of a pseudopolarographic experiment (see Figure 3), was –0.8 V. A deposition time of 20 minutes was used and the potential scan was carried out by differential pulse mode (scan rate 10 mV s^{-1}, pulse amplitude 50 mV, pulse frequency 5 s^{-1}) until a final potential of –0.15 V was reached. To strip out any residual quantity of metals deposited on the mercury film, the electrode was held at –0.18 V and rotated for 5 minutes.

In some cases (samples 8, 13, 1, 25, and 5, 7 under pack), titration data fitted the model of one class of ligands, within the limits of experimental error (detection limit for C_{L_1} is estimated as about 0.3 nM), thus the simple linear transformation given by equation 7 was directly applied to determine C_{L2} and K'_2. Figure 4 shows a typical titration curve and the related transformed plot fitting the one-ligand model.

For the remaining samples (the majority), the plot of $[Cu']/(Cu_t-[Cu'])$ versus $[Cu']$ assumed a curved shape, indicating the presence of more than one ligand (see Figure 5). In these cases, the model of two classes of ligands (one class of stronger ligands and one class of weaker ligands) given by equation 12 showed the best fit (Figure 5b displays a typical plot of residuals) and the parameters relative to the complexation were obtained by fitting the experimental data to equation 12 by the Marquart-Levenberg nonlinear fitting algorithm. Initial values for the parameters used in the fitting procedure were estimated by considering the two limiting situations obtained at low and high copper concentration, respectively. At low metal concentration, if $K'_1 >> K'_2$, complexes with stronger ligands (L_1) will be predominantly formed, so a plot of $[Cu']/(Cu_t-[Cu'])$ as function of $[Cu']$ approximately follows a straight line, typical for a system with one ligand[18], and the initial approximated values of C_{L1} and K'_1 can be calculated from the slope and the intercept obtained by application of the

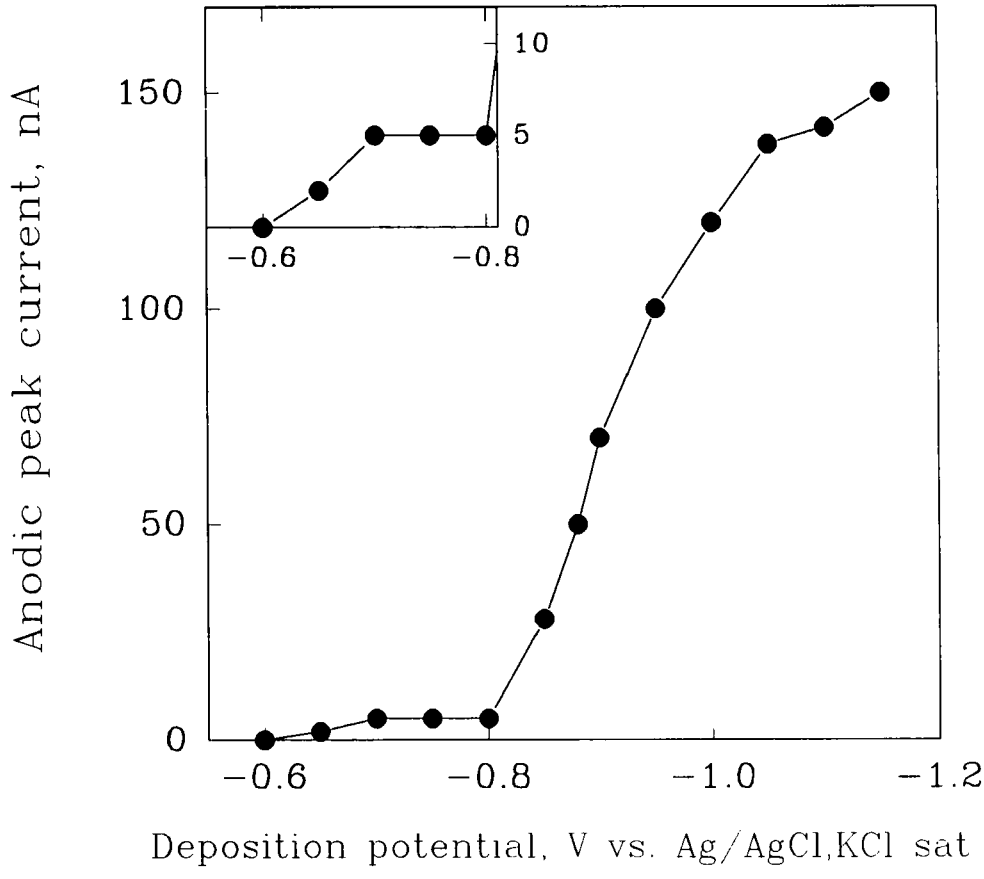

Figure 3 Pseudopolarographic experiment performed on a sample collected "ad hoc" in the North Adriatic Sea (3 miles offshore).

linearization procedure (equation 7) to the first few measurements of the titration. At high metal concentration (the final part of the titration), both ligands tend to be completely saturated, and the plot of $[Cu']/(Cu_t-[Cu'])$ versus $[Cu']$ tends to a straight line whose slope and intercept are $1/(C_{L1}+C_{L2})$ and $(C_{L1}/K'_1+C_{L2}/K'_2)/(C_{L1}+C_{L2})^2$, respectively[29]. So, by using the C_{L1} and K'_1, previously evaluated, and the experimental values for the latter slope and intercept, the initial values of C_{L2} and K'_2 can be obtained. It is interesting to note that the estimation of initial, approximated values of C_{L1} and K'_1 leads to overvaluation of C_{L1} and to underestimation of the K'_1; moreover the higher the C_{Cu}/C_{L1} ratio, the larger the error. In fact, the procedure neglects the part of the complexed copper which is bound to L_2, and the contribution of L_2 to the complexed copper $(Cu_t - [Cu'])$ in equation 12 becomes larger as the value of the C_{Cu}/C_{L1} increases. For example, the initial values of C_{L1} and K'_1 used for fitting data of sample 28 (1987–88 expedition), where the above ratio is 2.3, were 4.5 nM (11 times higher than the final value, i.e. 0.4 nM) and 5.6×10^9 M^{-1} respectively; for sample

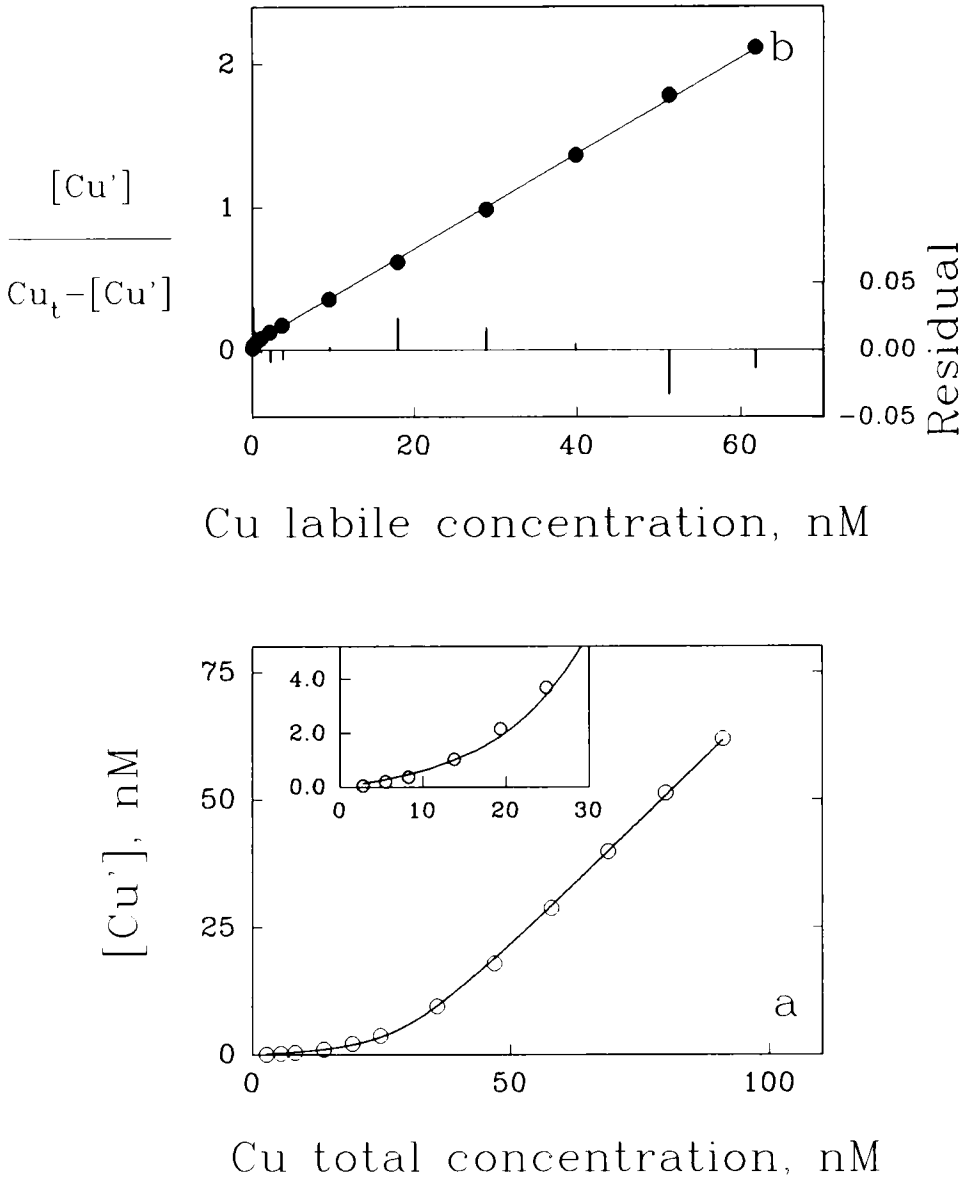

Figure 4 Titration curve (a) and transformed plot (b) for sample 5 collected under ice pack (1988–89 expedition) showing the fitting to one-ligand model.

37 (1987–88 expedition), where the ratio is 0.89, the initial values were 3.1 nM (1.7 times higher than the final value, i.e. 1.8 nM) and 1.6×10^{10} M^{-1}.

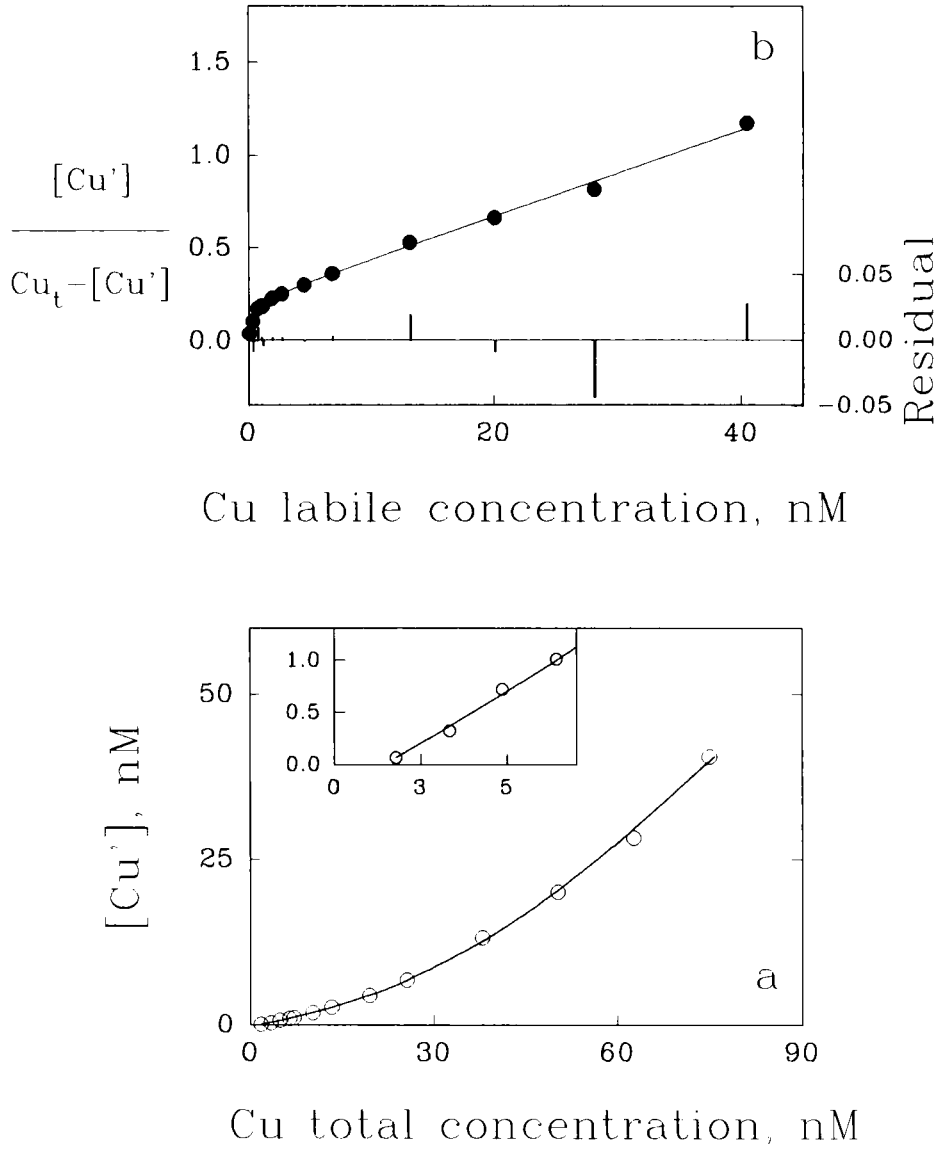

Figure 5 Titration curve (a) and transformed plot (b) for sample 16 (1987–88 expedition). The bargraph in (b) plots the difference between experimental data and fitting curve.

Measurements carried out on surface samples collected in the 1989–90 campaign (samples 32, 33, 34 and 35) do not show any signal corresponding to copper, either initially or even after the first few additions of metal to the sample were made (Figure 6). This indicates the practically complete complexation of the copper added, which depletes the

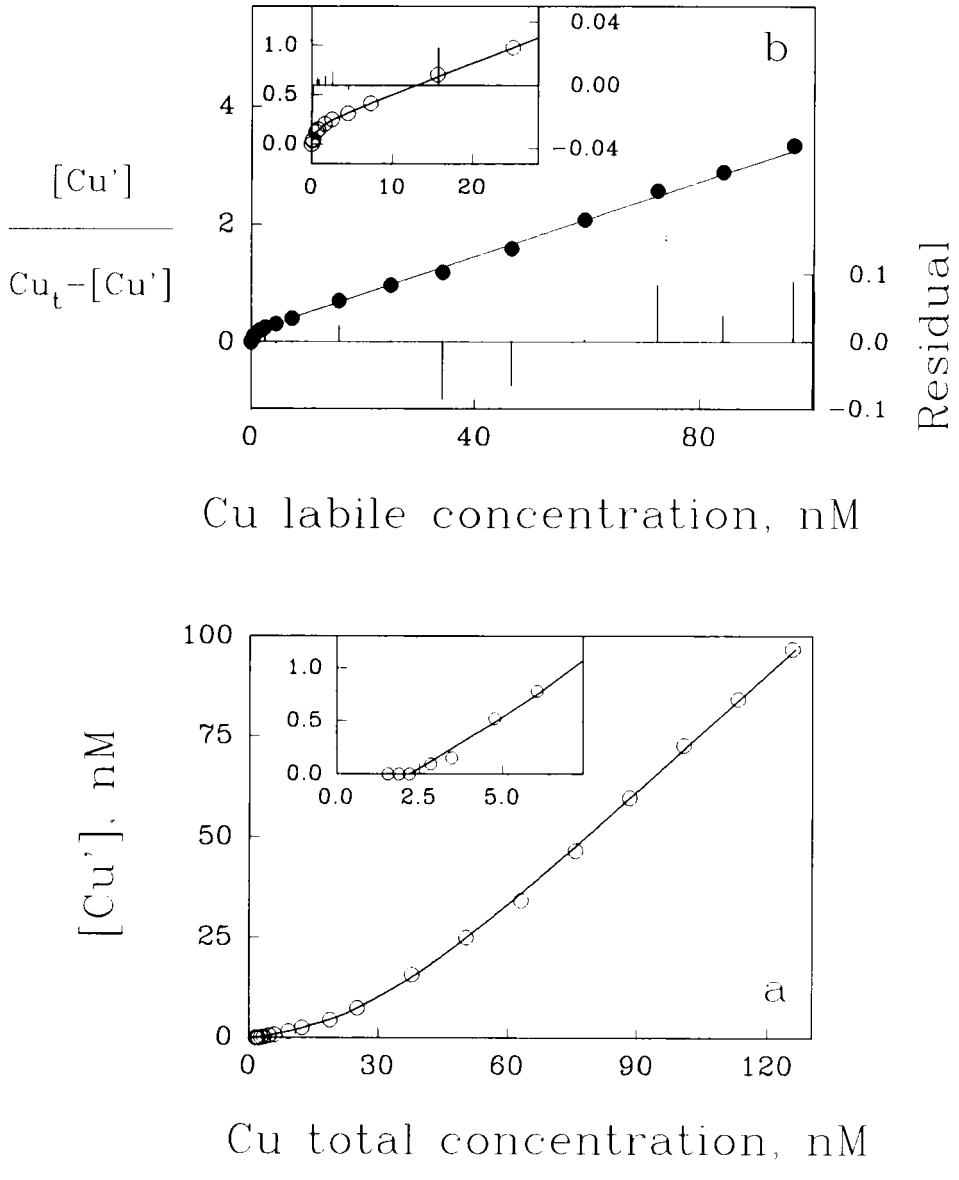

Figure 6 Titration curve (a) and transformed plot (b) for sample 35 (1989–90 expedition). The bargraph in (b) plots the difference between experimental data and fitting curve.

concentration of labile copper under the detection limit of the technique. Analogous observations were made by other scientists for samples collected in the photic layer of the North Pacific Ocean[16]. Since results of the fitting on these samples could be of reduced

reliability due to low precision of measurement in the first part of the titration, we also tried to apply the procedure suggested by Coale and Bruland[16]. In this procedure, the concentration of L_1 is evaluated on the basis of the total copper content (copper added plus the amount originally present in the sample) reached with the addition preceding that for which an ASV signal is detectable. For example, sample 32 did not show a detectable labile copper, either in the initial sample, when the total concentration of copper was 1.7 nM, or after two standard additions, when a total concentration of 3.1 nM was reached. After the third addition, a signal corresponding to labile copper was detectable, so, we considered the L_1 concentration to be 3.1 nM. This procedure could lead to an underestimation of C_{L1} as large as the first addition made to yield a non zero response (i.e. about 0.6 nM) and only an approximate value of K'_1 can be evaluated[16]; however, we observed a generally good agreement for the C_{L1} estimation between this second approach and the nonlinear fitting procedure (see Table 3). When the ASV labile fraction was initially undetectable, the [Cu'] value reported in Table 3 was evaluated through equilibrium calculations using the L_1 and L_2 concentrations and the related conditional stability constants (K'_1 and K'_2) obtained by the nonlinear fitting procedure. Calculations were performed by the TITRATOR program[38].

Measurements of repeatability for the speciation parameters carried out on sample 17 showed that the RSD (calculated from 5 measurements) was 49% for the ASV labile fraction at 0.08 nM, 69% for the concentration of L_1 at 0.39 nM, 12% for the concentration of L_2 and 48% for K'_2 at levels of 27 nM and 2.9×10^8 M^{-1} respectively. The K'_1 value, estimated by the nonlinear fitting, normally presents a very high standard deviation, so (except in one case which presents RSD < 100%) only the order of magnitude is reported in Table 3.

RESULTS AND DISCUSSION

Analytical results

The results of the analytical measurements performed on samples collected in the three Antarctic expeditions are reported in Table 3. Copper speciation in terms of total, labile and ionic concentration is given, together with the concentration of the two classes of ligands (C_{L1} and C_{L2}) and the related conditional stability constants (log K'_1 and log K'_2). All the speciation parameters were obtained by the titration experiment, except the ionic copper concentration, which was calculated (see below) from [Cu'] using literature values for α_{Cu}[39]. For cases in which the titration data fitted the one-ligand model, i.e. the concentration of the stronger ligand was below the detection limit of about 0.3 nM (see samples 8, 13, 1, 25 sea free from ice, and 5, 7 under the pack), the table reports only the values related to the weaker ligand (C_{L2} and log K'_2). For samples which did not show any copper signal (1989–90 campaign, surface seawater) [Cu'] was obtained by equilibrium calculations, while C_{L1} was estimated either by the fitting procedure or by the procedure based on the minimum copper amount before an ASV signal is detected (see detailed explanations above). Data referring to the total copper concentration represent the average of two measurements or more; other results are obtained from a single measurement (single titration experiment), repeated if necessary, in case of uncertainty.

Table 3 Speciation of copper, concentration of ligands and related conditional stability constants obtained in surface seawater samples of Terra Nova Bay (Antarctica).

Station	C_{Cu}, *nM*	*[Cu'], nM*	$p[Cu^{2+}]$	C_{L1}, *nM*	$\log K'_1$	C_{L2}, *nM*	$\log K'_2$
			1987–88 EXPEDITION				
8	4.3	0.59	10.5	udl[a]	—	14	8.79
9	2.8	0.06	11.5	1.8	16	31	8.23
10	2.4	0.03	11.8	1.7	14	39	8.26
12	1.6	0.08	11.4	0.7	14	37	8.44
13	1.4	0.16	11.1	udl	—	41	8.33
16	1.8	0.07	11.4	1.4	14	42	8.03
17	0.9	0.08	11.4	0.4	15	25	8.35
22	1.4	0.03	11.8	1.0	14	36	8.19
28	0.9	0.04	11.7	0.4	12	31	8.31
31	2.0	0.10	11.3	1.7	16	21	8.14
37	1.6	0.06	11.5	1.8	15	31	8.23
46	2.7	0.05	11.6	1.0	10.0	32	8.24
			1988–89 EXPEDITION				
			Samples collected after ice melting				
1	4.8	0.20	11.0	udl	—	35	8.15
2	2.9	0.15	11.2	1.8	15	31	8.26
3	2.3	0.02	12.0	1.0	16	24	8.26
4	1.7	0.10	11.3	1.1	13	32	8.23
5	2.5	0.04	11.7	1.1	14	34	8.66
6	1.1	0.02	12.0	0.5	14	25	8.80
7	1.8	0.06	11.6	1.7	12	23	8.12
			Samples collected under the pack				
5	2.8	0.04	11.6	udl	—	30	8.86
6	2.0	0.10	11.3	0.6	14	32	8.29
7	1.5	0.07	11.4	udl	—	36	8.70
			1989–90 EXPEDITION				
25 depth 20 m	1.9	0.16	11.1	udl	—	34	8.58
32	1.7	(8×10^{-6})[b]	15.5	3.8(3.1)[c]	14	33	8.69
33	2.1	(0.10)	11.5	2.0(3.6)	15	16	8.20
34	2.5	(3×10^{-6})	16.0	3.5(2.5)	15	31	8.66
35	1.6	(3×10^{-6})	16.0	2.2(2.2)	15	29	8.19

([a]) udl=under detection limit (about 0.3 nM).
([b]) In brackets values obtained by equilibrium calculations (Titrator program from Cabaniss, 1987[38]).
([c]) In brackets values estimated on the basis of the amount of copper added before the first ASV signal is detectable (see text).

Copper speciation

The total copper concentration ranges between 0.9 and 4.8 nM with a mean value of 2.1 nM (SD 0.9 nM) for the three campaigns. Considering that only 2 samples present values higher than 3 nM, we can say that no evident trend is visible in the distribution of total copper concentration in Terra Nova Bay, and excluding the same two samples, the mean concentration is 1.9 nM (SD 0.6 nM). These values are consistent with recent accepted data for coastal and shelf surface waters: Pacific region about 1.4 nM[40], Atlantic region about 4.0

nM[41], Arctic Ocean about 1.6 nM and Antarctic area about 2.5 nM[42,43]. Comparable values are also reported for open sea in the Atlantic Ocean, about 1.4 nM[44]. With reference to surface waters of Pacific open sea, the comparison is feasible with both eutrophic areas (comparable concentration, about 1.3 nM)[7] and oligotrophic areas (concentration about three times lower, about 0.5 nM)[16]. These comparisons of copper content seem to show an euthrophic situation for the studied area. However definite confirmation of this hypothesis requires knowledge of the vertical distribution of both copper and nutrients.

The labile fraction meanly represents about 4% of the total concentration for samples collected in the 1987–88 and 1988–89 expeditions, while samples of the 1989–90 season, excluding sample 25 collected at 20 m depth, present values under the detection limit and the bound fraction is higher than 99%. Biological and chemical (nutrients) measurements carried out during the 1987–88 and 1989–90 seasons in the same area confirm the substantially different oceanographic conditions of the two campaigns due to different seasonal evolution[45–48].

For most of the samples, titration data fitted the model of two classes of ligands. One class of stronger ligands, L_1, present at low concentration (the mean concentration for samples collected in water free from pack is 1.5 nM) and one class of weaker ligands, L_2, uniformly distributed in all of the examined area (the mean concentration is 31 nM, SD 6.8 nM) with a mean conditional stability constant of 2.8×10^8 M^{-1}, SD 1.8×10^8 M^{-1}. The concentration of the weaker ligands shows no variation for the 3 campaigns considered (mean concentrations were 32, 30 and 29 nM, respectively). On the other hand, the distribution of L_1 is strongly dependent on seasonal and spatial factors. Samples of the 1989–90 campaign, in particular, show a mean concentration (about 2.9 nM) which is more than double that of previous campaigns (about 1.2 nM). The spatial distribution of L_1 observed in Terra Nova Bay during the 1987–88 expedition (see Figure 7) presents considerable variability, as also observed for nutrients[45] and biological parameters[47], thus suggesting that the non-uniform distribution may be due to the differences in the hydrological characteristics of the area examined.

During the summer of 1988–89 some samples (5, 6 and 7) were collected before and after the pack melting; the two kinds of samples show no marked difference as regards the concentrations of copper and the weaker ligand L_2, but the mean concentration of L_1 is at least tripled after the melt.

A number of samples (8, 13, 1, 25, and 5, 7 under pack) do not show any presence of L_1 given the sensitivity of the voltammetric technique. In a few cases this observation can be considered as a logical consequence of the complete saturation of L_1 by copper before the beginning of titration due to the high metal concentration in the sample (see e.g. samples 8 and 1). In fact, this situation makes it impossible to study and detect the stronger class of ligands. A second interpretation of these results can be linked to the non-homogeneous characteristics of the area studied; this hypothesis is confirmed, as reported above, by the observations of other researchers operating in the same area during the 1987–88 expedition, regarding biological activity[47] and water circulation[49]. Moreover, we cannot exclude that both the suppositions of ligand saturation by copper and the marked dishomogeneity of the area may contribute to the same phenomenon. Finally, a particular consideration should be made for sample 25, collected during the last expedition at a depth of 20 m. It is known[7,16]

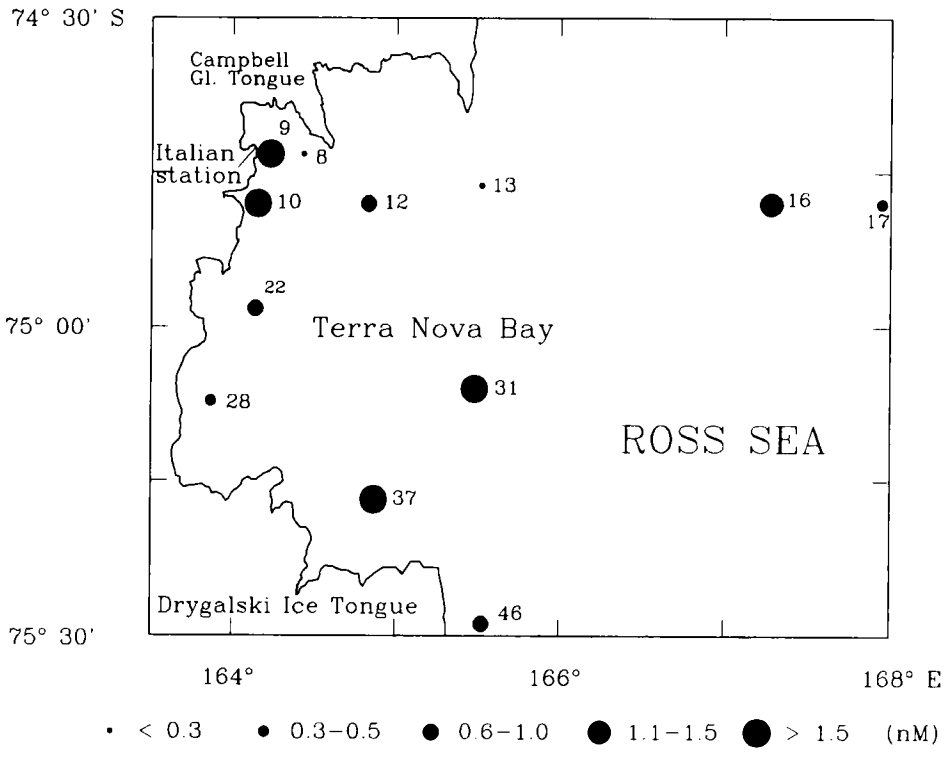

Figure 7 Spatial distribution of the stronger ligand concentration, C_{L1}, in surface water of Terra Nova Bay (1987–88 expedition).

that the vertical distribution of the strong ligand L_1 shows a maximum at approximately the same depth as the maximum primary production, after which it rapidly decreases to the zero value. In station 25 the ^{14}C assimilation is concentrated in the initial 2–3 m depth[50], so the absence of L_1 in this sample, collected at 20 m depth, seems to be in agreement with the observations of the quoted researchers.

Literature data show that detection of two classes of ligands complexing copper is becoming common, the concentration for L_1 varying between 1.8 and 36 nM and L_2 ranging between 8 and 99 nM[7,9,16,34]. It appears that our results (average C_{L1}=1.5 nM and C_{L2}=31 nM) are consistent with ligand concentration detected in open ocean, i.e. about 4 and 30 nM for C_{L1} and C_{L2}, respectively.

As regards the weak ligand L_2, good agreement between the concentration values measured in very different geographical areas can be observed. The only exception concerns the results reported by Coale and Bruland[16], who found values about one third of ours, probably because their measurements were exploring a lower titration range (0–30 nM) than ours (0–90 nM), and the results reported by van den Berg[51], obtained by applying the technique based on absorption of inorganic copper on MnO_2.

The concentration for ligand L_1 reported in the literature, presents a larger variability than that of L_2, due to differences in the characteristics of the areas studied, but, also probably because of differences in the methodologies used. We can observe that our results are in agreement with values measured in open ocean using electrochemical methods[7,18]. The mean value for the conditional stability constant K'_1 obtained by us is markedly higher than the value reported by these latter scientists. This is probably because of the particular sensitivity of this parameter to the precision of measurements, as pointed out by other authors[16] as well.

Ionic copper and bioavailability

As mentioned above, the bioavailability of copper is related to its ionic activity, the evaluation of which requires an understanding of the metal complexation with organic ligands, as well as a knowledge of inorganic speciation. In the present context, the concentration of Cu^{2+} can be estimated from the experimental concentration of inorganic copper, [Cu′], and from the coefficient for the inorganic side reactions, α_{Cu}. The copper activity can then be computed through the activity coefficient of copper in seawater conditions.

Byrne and Miller[52] showed that the dominant inorganic species of Cu (II) are carbonate complexes; Byrne et al.[39] also reported a dependence of α_{Cu} on pH and temperature. The α_{Cu} = 24 calculated by Byrne and Miller[52] for seawater at pH 8.2 and T = 25°C must be corrected for the pH and temperature of our samples in the experimental measuring conditions (natural pH, 20°C). Using thermodynamic data from Byrne et al.[39] we can calculate a value of α_{Cu}=18 at pH 8.2 (samples of 1987–88, 1988–89 under pack, 1989–90 sample 25), α_{Cu}=23 at pH 8.3 (samples of 1988–89 after melt) and α_{Cu}=26 at pH 8.4 (samples 32–35 of 1989–90 expedition).

As a consequence of the observed values of the complexation parameters we can note that the concentration of ionic copper is strongly affected by the concentration of L_1; in fact as the concentration of L_1 increases from a mean value of 1.2 nM for the 1987–88 expedition, to a value of 2.9 nM for the surface samples collected during 1989–90 expedition, a decrease of $[Cu^{2+}]$ is observed ($p[Cu^{2+}]$ varies from a mean of 11.4 to 14.8). By considering an activity coefficient of 0.22[52] we can calculate mean activities for Cu^{2+}, expressed as $p\{Cu^{2+}\}$, of 12.1 and 15.5 respectively. It is interesting to note that the latter activity can be sufficiently low so as to limit the growth of certain organisms as discussed in the introductory section. However, it should be noted that the mean value relating to the 1989–90 expedition was computed from the equilibrium data and not from the experimental measurement of [Cu′], so the reliability of this result could be questioned if one considers (see above) the possible over-estimation of K'_1 which, in turn, leads to an underestimation of $\{Cu^{2+}\}$. However, even if we use the K'_1 value reported in the literature[9,16,18] for the calculations, i.e. 4.5×10^{12} M^{-1}, we obtain a mean value of 13.9 for $p\{Cu^{2+}\}$. Therefore, also in this case the low copper activity detected in the 1989–90 expedition may constitute a limiting factor for biological activity in the studied area, or a selective force acting on the species distribution of the phytoplankton communities.

CONCLUSION

Although there is experimental evidence in different areas leading to the hypothesis regarding the biological origin of ligands complexing copper, at present the problem is not completely clear, especially as regards identification of the ligands and their source and the effect of complexation on copper distribution. More conclusive observations can be obtained from a systematic study which attempts to detect a correlation between the complexation data and primary production, or other parameters which define biological activity during the entire phytoplankton bloom.

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