Relations between free copper and salinity, dissolved and particulate organic carbon in the Oosterschelde and Westerschelde, Netherlands

L.J.A. Gerringa *, H. Hummel, T.C.W. Moerdijk-Poortvliet

Netherlands Institute for Ecology, Center for Estuarine and Coastal Ecology, P.O. Box 140, 4400 AC Yerseke, Netherlands

Abstract

In a previous investigation, relatively simple relations were found to estimate free Cu, and hence a good approximation of bio-available Cu, at two sites in estuarine waters of the Dutch Oosterschelde and Westerschelde. In the present study, these relations are validated for the remainder of the two sea arms. In the Westerschelde the relation between the concentration of a dissolved organic ligand ($K = 10^{13}$), DOC (dissolved organic carbon) and salinity is valid in a range of salinities at least from 10 up to 25 to 30. This ligand group is primarily riverine and partly estuarine. It is, as far as we know, the strongest ligand for Cu, and its concentration dominantly affects the free Cu ion concentrations, which are around 50 fM. At high salinities, dilution and mineralisation diminish the riverine member of the ligand group too much and the relation with salinity and DOC is no longer valid. In the Oosterschelde the relations between POC (particulate organic carbon) and particulate Cu and between POC and the ratio of particulate and dissolved Cu are valid for the western, central and eastern compartments. Since equilibrium was assumed to exist between dissolved and particulate Cu, free Cu can be calculated from POC and particulate Cu concentrations. Due to the large adsorption capacity of POC, the Oosterschelde is with respect to Cu a very well-buffered system with $[Cu^{2+}]$ of 20 fM. © 1998 Elsevier Science B.V. All rights reserved.

1. Introduction

Measuring Cu speciation is a time-consuming and relatively complicated analysis. For studies on accumulation and effects of Cu on biota it is essential to know the speciation of Cu, since it is assumed that the bio-availability of Cu is determined by the free concentration of the Cu cation (Sunda and Guillard, 1976). So far many investigations on exposure and toxicity of Cu on biota have been performed using total dissolved concentrations (Bordin et al., 1992; Hoare et al., 1995a,b). However, in a previous investigation Gerringa et al. (1996a,b) were able to estimate Cu speciation by means of a set of relatively simple chemical parameters including salinity, particulate organic carbon (POC) and dissolved organic carbon (DOC) (Table 1). These relations were based on data collected at only two sampling stations. In the present study we present a wider validation of these relations in the geographical sense. To this end, Cu speciation was measured together with the parameters influencing Cu speciation at several sam-

* Corresponding author. Present address: Netherlands Institute for Sea Research, P.O. Box 59, 1790 AB Den Burg, The Netherlands; E-mail: loes@nioz.nl

Table 1
Relations found by the multiple regression with $R^2 > 0.5$ (from Gerringa et al., 1996b)

(1)	$[Cu]_p = -0.144 + 0.0456\,POC$	$R^2 = 0.947$	OS
(2)	$Q = 0.101 + 0.0067\,POC - 0.0101\,PTN$	$R^2 = 0.922$	OS
(3)	$L_ta = 583 - 29.2\,sal + 12\,Ch\alpha_c + 0.95\,[Zn]_d$	$R^2 = 0.722$	WS
	$(L_ta = 883 - 39.4\,sal + 10\,Ch\alpha_c$	$R^2 = 0.629$	WS)
(4)	$L_ta = -52.4 + 1.0944\,DOC$	$R^2 = 0.522$	OS
(5)	$L_tc = 379 - 24.6\,sal + 1.512\,PTN + 14.3\,CuS$	$R^2 = 0.751$	WS
	$(L_tc = 434 - 20.7\,sal + 1.694\,PTN$	$R^2 = 0.715$	WS)
(6)	$L_tc = -96.2 + 0.4488\,DOC + 0.6\,[Zn]_d$	$R^2 = 0.586$	OS
	$(L_tc = -33.4 + 0.3108\,DOC$	$R^2 = 0.393$	OS)
(7)	$L_tc = 117 + 0.4296\,DOC - 6.3\,sal$	$R^2 = 0.704$	WS + OS
(8)	$[Cu]_p = 58.2 + 0.0408\,POC - 1.9\,sal$	$R^2 = 0.784$	WS + OS

For Eqs. 1–6, data from monitoring during 1993 were used. For Eqs. 7 and 8, data from both estuaries were used. Variables which did not seem chemically meaningful were omitted from Eqs. 3, 5 and 6, and the resulting equations are given in brackets.
Alpha to enter and alpha to remove were set to 0.1 (90% confidence interval).
Units for all variables above as given in Tables 2 and 3.
DOC = dissolved organic carbon; POC = particulate organic carbon; PTN = particulate total N; $[Cu]_p$ = particulate Cu; Q = ratio of particulate and dissolved Cu; L_ta = relatively weak ligand group, determined with DPASV; L_tc = relatively strong ligand group, determined with DPCSV; CuS = Cu extracted from Sep-Pak C_{18} cartridges; $[Zn]_d$ = dissolved Zn; $Ch\alpha_c$ = marine chlorophyll-a; sal = salinity.
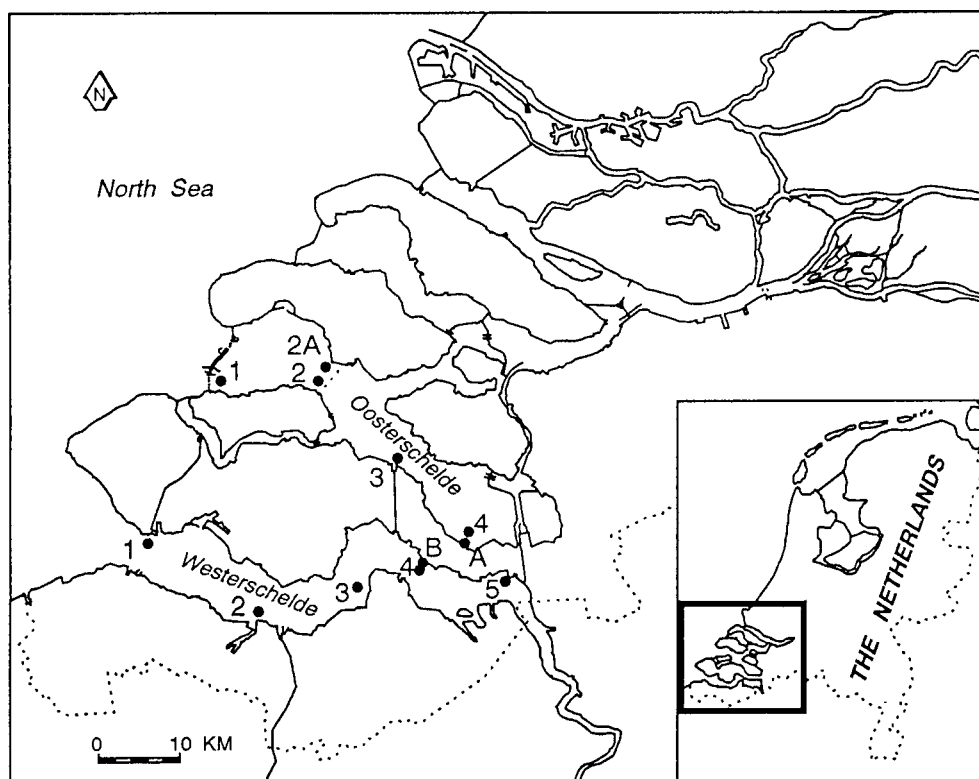

Fig. 1. Map of the southwestern part of the Netherlands, indicating the location of sampling stations. Station 2A was only sampled in February 1993. Stations numbered 4 in the Oosterschelde and Westerschelde are as close as possible to the monitoring stations (A and B). In November 1992, sampling was performed over a full tidal cycle at the Oosterschelde and Westerschelde stations 4.

pling stations in a marine tidal sea arm, the Oosterschelde, and a brackish estuary, the Westerschelde (Fig. 1).

Since the construction of a storm surge barrier in 1987, the Oosterschelde has become a marine tidal basin without a distinct salinity gradient. The mean freshwater influx is around $25 \text{ m}^3 \text{ s}^{-1}$. Current velocities are low and, consequently, the turbidity is relatively low (4 m Secchi disc depth in summer). The residence time varies between 10 days in the western part and 100 to 200 days in the eastern parts. The freshwater load has decreased to less than 1% of the tidal volume; hence the system has changed from an estuary to a tidal bay. Salinity has gone up and water quality improved, while nutrient concentrations have decreased (Wetsteyn and Bakker, 1991; Smaal and Nienhuis, 1992; Wetsteyn and Kromkamp, 1994). Comparison of Cu and POC concentrations from Valenta et al. (1986) from before the completion of the storm surge barrier with more recent data from Gerringa et al. (1996a,b) indeed shows substantial decreases. The exchange with the North Sea has also decreased. Suspended-matter concentrations have dropped below 10 mg dm^{-3} in the eastern and northern sections and no seasonal pattern is observed (Wetsteyn and Bakker, 1991; Smaal and Nienhuis, 1992; Wetsteyn and Kromkamp, 1994). The previously assessed relations in Gerringa et al. (1996a,b) were obtained for the sampling site in the eastern section (No. 4 in the Oosterschelde, Fig. 1). Two highly significant relations were found between (1) particulate Cu ($[Cu]_p$) with particulate organic carbon (POC), and (2) the ratio of particulate and dissolved Cu (Q) with POC ($R^2 > 0.9$; Table 1). Equilibrium was assumed between dissolved and particulate Cu at the eastern sampling site (No. 4, Fig. 1) in the Oosterschelde. This assumption was based on the high statistical significance of the above-mentioned relations and on measurements of the adsorption capacity of the suspended matter. Expressed as sites of mol Cu per mol organic C, the adsorption capacity was 0.01316 molequivalents of Cu/mol C with a conditional stability constant of $10^{11.35}$ (Gerringa et al., 1996a,b). Chemical equilibrium calculations showed that these binding characteristics explained the division of Cu over the dissolved and particulate phases and thus equilibrium between the phases was assumed. It was

concluded that the key factor for the distribution over the different Cu fractions was the adsorption onto organic particles. Hence the concentration of ionic Cu ($[Cu^{2+}]$) could be calculated with the two relations mentioned above and the adsorption characteristics.

The Westerschelde is one of the most polluted estuaries in Western Europe. It is a well-mixed estuary with only small and local vertical salinity gradients. The residence time in the estuary is around 60 days. The high turbidity zone is upstream at a salinity (Sal) of 5. Due to the high organic load of the river Scheldt, the upper parts of the estuary consist of anaerobic waters. Increasing oxygen concentrations are observed between salinities of 5 and 15, depending on the season (Duinker et al., 1982; Zwolsman and Van Eck, 1993). The mid-estuarine maximum of dissolved metals due to oxidation of Mn is between salinities of 15 and 25 (Duinker et al., 1982; Wollast, 1988). Particles of riverine origin are mostly co-precipitated in the high-turbidity zone where oxidation processes take place. Further out, the suspended matter is predominantly of marine origin (Regnier and Wollast, 1993). Particulate Cu is mainly associated with POC (Regnier and Wollast, 1993; Monteny et al., 1993) as was also found by Gerringa et al. (1996a,b) when pooling all data (see Eq. 8 in Table 1). Monteny et al. (1993) found highly significant particulate Cu/POC relations ($r = 0.964$) in data from 1982. Dissolved Cu clearly shows a mid-estuarine maximum around $S = 20$ due to oxidation and desorption processes (Monteny et al., 1993; Zwolsman and Van Eck, 1993). Dissolved Cu is predominantly associated with organic ligands (Van den Berg et al., 1987; Regnier and Wollast, 1993; Monteny et al., 1993).

The sampling site during the previous monitoring program of Gerringa et al. (1996a) was Waarde (No. 4 in the Westerschelde, Fig. 1). Relations were found for two distinct dissolved organic ligand groups (L_ta and L_tc) which together control the free-Cu concentration. The first group (L_ta a relatively weak ligand group, measured with anodic stripping voltammetry, ASV) exhibited some empirical dependence on salinity and marine Cha, the second group (L_tc a relatively strong ligand group, measured with cathodic stripping voltammetry, CSV) on salinity and PTN (particulate total nitrogen) (Gerringa et al., 1996a,b; see also Table 1).

In view of the hydrographic characteristics and differing particle dynamics and characteristics, it can be expected that the previously assessed relations in the Oosterschelde should be valid for more than just the eastern part, whereas for the Westerschelde an extrapolation to other sites may be questionable.

2. Materials and methods

Samples were taken in November 1992 and February 1993 along the Oosterschelde and Westerschelde from west to east (Fig. 1). They were taken at high tide upstream from the research vessel *Luctor* with a telescopic fishing rod to which a polyethylene sampling bottle was attached just below the surface. In November 1992 the tidal influence was investigated at the sampling stations where the previous monitoring by Gerringa et al. (1996a,b) was done (A and B, Fig. 1). Samples for Cu speciation measurements were taken every two hours throughout one tidal cycle and every hour for the other parameters. In February 1993 an additional location in the Oosterschelde (station 2A, Fig. 1) was sampled. This site was an ammunition depot during the Second World War.

All materials used during sampling for metal analysis were acid cleaned (at least 2 weeks in $HNO_3/MilliQ = 1:5$). Sample handling and analysis of metals and speciation measurements took place in a clean room and MilliQ water (≥ 18.2 Ω^{-1}) was used, unless otherwise stated. The samples were filtered immediately on board under reduced nitrogen pressure through an acid-cleaned 0.45-μm cellulose–nitrate filter. Sample bottles containing the dissolved phase were removed before the filter became dry.

Speciation of Cu was done by differential pulse anodic stripping voltammetry (DPASV) (Kramer, 1986), differential pulse cathodic stripping voltammetry (DPCSV) (Campos and Van den Berg, 1994) and by reverse-phase liquid chromatography (Sep-Pak C_{18}, Mills and Quinn, 1984). A hanging mercury drop electrode was used (PAR-EEG 303 electrode stand with a 384B analyzer) in the voltammetric determinations.

For DPCSV, salicylaldoxime (SA) was used at a concentration of 10^{-5} M (Campos and Van den Berg, 1994). The alpha-factor (α) for the inorganic complexation was assumed to be 50. The α for the added SA represents the centre of the detection window of the method and is (depending on the value of salinity) around 130 000 for the Oosterschelde samples and between 130 000 and 232 000 for the Westerschelde samples. The ligands measured by this method are marked with a 'c' (cathodic). They had conditional stability constants between 10^{15} and $10^{12.5}$ in the Oosterschelde, and between $10^{13.4}$ and $10^{12.3}$ in the Westerschelde (Gerringa et al., 1996a,b).

For DPASV, a value of $10^{2.6}$ was assumed for α (Van den Berg and Donat, 1992). According to these authors the upper and lower limits of the detection window are one order of magnitude above and below α, respectively. The ligands measured with DPASV are the ones which match the extent of binding by this analytical window. These ligands, which are notated with an 'a' (anodic), showed conditional stability constants between $10^{10.2}$ and $10^{8.4}$ for the Oosterschelde, and between $10^{10.4}$ and $10^{8.2}$ for the Westerschelde (Gerringa et al., 1996a,b).

The ligand concentration is expressed as nmol equivalents of Cu per dm^3 (neq dm^{-3}), since the actual measurement consists of measuring the concentration of Cu that can be complexed by the organic ligands.

Dissolved Cu and Zn were measured in acidified samples (pH = 2) by DPASV (standard error (s.e.) of 3 nM for dissolved Cu; s.e. of 10 nM for dissolved Zn). Particulate Cu and Cu extracted by Sep-Pak cartridges were measured by atomic absorption spectrophotometry with Zeeman background correction (s.e. of 2 nM for particulate Cu; s.e. of 1.1 nM for Sep-Pak Cu).

DOC was determined by colorimetric titration of released CO_2 (s.e. of 17 μM C dm^{-3}; Schreurs, 1978).

Particulate organic carbon (POC) and particulate total nitrogen (PTN) were measured on a Carlo Erba nitrogen/carbon analyzer (s.e. of 3% for POC and PTN; Nieuwenhuize et al., 1994).

Chlorophyll was measured using high-performance liquid chromatography according to Brown et al. (1981) (s.e. 8%). Marine chlorophyll was calculated from total chlorophyll minus 5 times chlorophyll-*b*. The ratio chlorophyll-*a*/chlorophyll-*b* fluctuates. The choice of a constant value for this ratio is

arbitrary, and '5' was chosen in this study, according to Senger et al. (1993).

Gerringa et al. (1996a,b) derived the relations shown in Table 1 by automated forward stepwise multiple regression of the data. The regression starts with one variable. The next variable, which is added to the regression, is the one which produces the largest increment in R^2. 'Stepwise' means that after a certain number of variables have been added, the data are scanned for any added variables that should now be deleted using the following criteria. The minimum tolerance used for entry of parameters into the model was set to 0.1, i.e. a significance level of 90% (Sokal and Rohlf, 1995).

Values for the dependent variables were calculated from the multiple regressions and the independent variables measured at the sampling sites along the two estuaries. By means of a t-test, the 95% confidence interval of the predicted standard error was set for each estimated dependent variable. We compared the measured values of the dependent variables with these intervals.

3. Results

In the Oosterschelde, the salinity increases slightly from east to west. The concentrations of dissolved and particulate Cu, dissolved Zn, POC, PTN and Cha, increase in the same direction (Table 2), while the concentrations of both the relatively weak ligand group ($L_t a$) and the relatively strong ligand group ($L_t c$) decrease (Table 3).

In the Westerschelde, the concentrations of DOC, POC, dissolved and particulate Cu and both ligand groups decrease with increasing salinity (Tables 2 and 3). In November, a maximum of dissolved Cu at sampling site WS 4, situated around the mid-estuarine maximum of Mn (Zwolsman and Van Eck, 1993), coincided with a minimum in particulate Cu and a maximum in the concentration of the relatively strong ligand.

In February the salinities of the sampling sites in the Westerschelde were lower than in November (Table 2), probably because of the higher run-off by the river Scheldt. Particulate Cu and the concentration of the relatively strong ligand were also higher in February than in November at WS3, 4 and 5.

In November, samples were taken over 13 h at the sampling sites of the monitoring program (numbers 4 near A and B, Fig. 1) to estimate the influence of the tidal cycle on concentrations. However, no trend could be discovered in the data. This was to be expected for the well-mixed Oosterschelde, but is somewhat surprising for the Westerschelde. Van Alsenoy (1993), however, also did not observe a tidal trend in her metal data from the Westerschelde.

With respect to the validation of the equations obtained in the previous survey, which was limited to one sampling station per estuary, one equation for the Westerschelde and four equations for the Oosterschelde could be applied to other parts of the estuaries (Table 1; Figs. 2 and 3).

Using Eq. 1, particulate Cu (Cu_p) agreed with 8 out of 9 measured values of Cu_p (Fig. 2A). Using Eq. 8, all measured concentrations were within the 95% confidence intervals, although the trend in the measured values could not be seen in the predicted values (Fig. 2B). The predicted ratio of particulate and dissolved Cu (Q in Eq. 2, was confirmed by 8 out of 9 measured values.

For the Oosterschelde, the validity of Eq. 4, as a tool to predict the concentration of organic ligands measured by DPASV ($L_t a$), was confirmed for all sampling stations during both periods (Fig. 2D).

For the Westerschelde, all but one of the organic ligand concentrations measured by DPCSV ($L_t c$) were within the probability intervals for the values predicted by Eq. 7 (Fig. 3).

4. Discussion

Five successfully validated equations (Figs. 2 and 3) can, without complicated and time-consuming analyses, be used to obtain a fair estimate of the Cu speciation and fractionation from parameters such as POC, DOC and salinity (Table 1).

4.1. Oosterschelde

The $L_t a$ concentrations in the Oosterschelde (Fig. 2D) predicted by Eq. 4 had very large 95% confidence intervals due to a low correlation coefficient ($R^2 = 0.522$). This equation is therefore of limited value for predicting the weaker-ligand con-

Table 2
Measured variables in Oosterschelde and Westerschelde in November 1992 and February 1993

Sample	Sep-Pak Cu (nM)	diss. Cu (nM)	part. Cu (nM)	diss. Zn (nM)	DOC (µM)	POC (µM)	PTN (µM)	Cha (µg/l)	sal	pH
November 1992										
OS1	2.3	17.5	8	50	122	99	11.4	1.2	32.15	7.60
OS2	2.6	7.6	3	29	133	60	6.4	0.9	32.20	7.61
OS3	2.4	6.3	2	26	156	39	3.6	0.7	31.32	7.60
OS4	3.1	6.0	1	39	162	34	3.6	0.5	30.96	7.58
WS1	4.9	7.6	4	77	155	70	7.9	1.8	30.08	7.63
WS2	3.7	4.4	13	69	193	89	10.0	2.6	26.04	7.58
WS3	7.1	16.2	17	89	236	171	17.1	4.0	22.64	7.59
WS4	9.4	28.8	11	144	308	114	12.1	3.4	18.16	7.54
WS5	8.4	13.8	32	131	378	103	11.4	3.3	11.78	7.31
OS4 1	3.0	5.0	3	36	169	56	5.0	0.6	30.96	7.57
OS4 2					161	52	5.0	0.7		7.57
OS4 3	2.9	11.2	3	42	152	53	5.7	0.6	30.84	7.56
OS4 4					159	47	4.3	0.8		7.56
OS4 5	3.0	5.8	2	17	158	48	5.0	0.6	31.15	7.56
OS4 6					177	32	2.9	0.5		7.57
OS4 7	3.4	2.4	2	36	178	34	3.6	0.6	30.91	7.60
OS4 8HW					168	31	2.9	0.6		7.60
OS4 9	3.4	7.7	1	37	171	32	3.6	0.6	30.98	7.62
OS4 10					159	27	2.9	0.6		7.63
OS4 11	3.2	12.1	1	72	162	28	2.9	0.6	30.91	7.64
OS4 12					178	33	3.6	0.5		7.64
OS4 13	3.3	6.0	1	23	161	40	4.3	0.5	31.01	7.65
OS4 14					167	38	3.6	0.5		7.67
WS4 1	9.4	25.2	21	174	312	82	9.3	2.8	16.16	7.48
WS4 2					313	75	7.9	3.1		7.47
WS4 3	9.8	22.2		139	339	66	7.9	2.8	16.14	7.47
WS4 4					311	90	9.3	3.0		7.48
WS4 5	9.3	20.0	10	119	360	69	7.9	3.0	17.39	7.50
WS4 6					334	149	15.7	3.7		7.55
WS4 7HW	9.1	24.2	16	149	248	105	12.1	3.8	20.71	7.58
WS4 8					257	72	7.9	3.0		7.58
WS4 9	9.1	25.5	21	109	298	120	12.1	3.5	19.50	7.57
WS4 10					293	109	10.7	3.2		7.53
WS4 11	11.3	13.1	29	99	303	122	12.9	3.3	16.16	7.52
WS4 12					301	161	17.9	3.1		7.52
WS4 13	11.4	18.4		142	309	123	12.9	2.6	15.89	7.51
WS4 14					341	95	11.4	2.1		7.51
February 1993										
OS1	1.9	9.4	5	71	123	70	7.9	2.1	31.89	7.84
OS2	2.7	6.9	3	72	157	32	3.6	1.7	31.17	7.85
OS2A	2.6	10.7	4	50	106	46	5.0	1.8	31.25	7.86
OS3	2.9	4.1	4	34	122	58	5.7	1.3	30.61	7.86
OS4	3.2	6.9	2	58	138	37	4.3	1.4	30.32	7.87
WS1	3.3	9.6	4	108	199	47	5.0	1.7	25.62	7.79
WS2	4.9	10.2	19	224	251	125	13.6	2.9	21.27	7.73
WS3	6.2	16.5	24	120	285	137	13.6	2.8	18.92	7.70
WS4	9.1	23.0	28	140	345	137	14.3	2.4	15.70	7.65
WS5	7.5	42.3	40	276	406	306	24.3	0.9	10.18	7.51

At stations OS4 and WS4, samples were taken throughout a tidal cycle (No. 1 at high tide, followed by sampling every hour).

Table 3
Concentrations and stability constants of the two classes of Cu-binding ligands

	log K_a	$L_t a$ [a]	log K_c	$L_t c$ [a]
November 1992				
OS1	10.29 (9.74–10.83)	75 (62–89)	14.23 (13.75–14.72)	24 (22–27)
OS2	10.08 (9.61–10.55)	81 (67–94)	14.41 (13.52–15.5)	56 (52–61)
OS3	9.70 (9.39–10.01)	57 (50–64)	13.99 (12.74–15.23)	22 (16–27)
OS4	9.07 (8.69–9.46)	194 (139–250)	13.58 (13.07–14.09)	60 (51–70)
WS1	9.52 (9.37–9.61)	140 (125–155)	14.25 (13.59–14.91)	33 (29–36)
WS2	9.48 (9.1 –9.85)	53 (41–65)	13.89 (13.57–14.22)	55 (50–60)
WS3	9.35 (8.55–10.16)	47 (31–62)	13.96 (13.77–14.15)	79 (75–83)
WS4	9.45 (8.48–10.42)	138 (93–183)	13.12 (12.98–13.27)	163 (153–173)
WS5	9.21 (9.09–9.34)	484 (450–519)	13.11 (13.02–13.21)	163 (154–172)
KR1	9.39 (9.04–9.73)	63 (51–76)		
KR3	9.44 (9.18–9.71)	96 (78–114)		
KR5	9.48 (9.24–9.72)	127 (104–150)		
KR7	9.55 (9.3 –9.8)	66 (57–74)		
KR9	9.2 (8.97–9.42)	94 (83–104)		
KR11	9.9 (9.68–10.12)	94 (83–104)		
KR13	9.58 (9.47–9.7)	102 (96–109)		
WA1	8.98 (8.62–9.34)	194 (148–201)		
WA3	8.97 (8.64–9.3)	171 (133–209)		
WA5	8.99 (8.72–9.25)	251 (206–297)		
WA7	9.97 (9.4 –10.53)	115 (91–140)		
WA9	8.88 (8.61–9.14)	252 (208–296)		
WA11	9.4 (9.2 –9.6)	231 (206–256)		
WA13	8.76 (8.43–9.09)	265 (198–333)		
February 1993				
OS1	9.77 (9.54–10.0)	53 (43–62)		
OS2	9.6 (9.39–9.81)	90 (75–104)		
OS2a	9.66 (9.44–9.88)	72 (59–85)		
OS3	9.18 (9.0 –9.36)	129 (113–146)		
OS4	9.32 (9.1 –9.54)	123 (104–141)	14.57 (13.81–15.34)	9 (7.6–10.4)
WS1	9.64 (8.86–10.42)	60 (45–76)	14.78 (14.33–15.22)	19 (16–21)
WS2	9.29 (8.84–9.74)	97 (78–115)	13.87 (13.73–14.01)	50 (46–53)
WS3	9.24 (8.84–9.65)	252 (189–315)	13.19 (13.11–13.27)	109 (104–114)
WS4	8.77 (8.44–9.1)	158 (112–205)	12.94 (12.74–13.14)	203 (182–225)
WS5	9.21 (9.03–9.39)	324 (290–358)	13.12 (12.98–13.25)	291 (266–317)

The 95% confidence intervals of the estimates are given in brackets; estimation according to the Langmuir isotherm with non-linear regression (Gerringa et al., 1995). The strong-ligand group was not determined in the samples taken over a tidal cycle (OS4 and WS4 1–13). In February, the strong ligand was below the detection limit for sampling stations 1, 2, 2A and 3 in the Oosterschelde.
[a] Lt in nmol equivalents of Cu/l.

centrations, although Fig. 2D shows that especially in February the estimated values are very close to the measured values.

For the Oosterschelde, particulate Cu concentrations predicted by Eq. 1 had narrower 95% confidence intervals ($R^2 = 0.947$) than predicted by Eq. 8 ($R^2 = 0.784$) Fig. 2B). Yet, since in almost all cases the measured particulate Cu at the sampling stations was within the confidence intervals of both Eqs. 1 and 8 the particulate Cu for the whole basin can be estimated from both equations.

Based on measurements of the adsorption characteristics of particulate matter together with the highly significant relations between particulate Cu and POC, Gerringa et al. (1996a,b) assumed equilibrium between particulate and dissolved Cu at sam-

pling station 4 of the Oosterschelde. Eq. 2 was validated for the other sampling points in the Oosterschelde and thus equilibrium between particulate
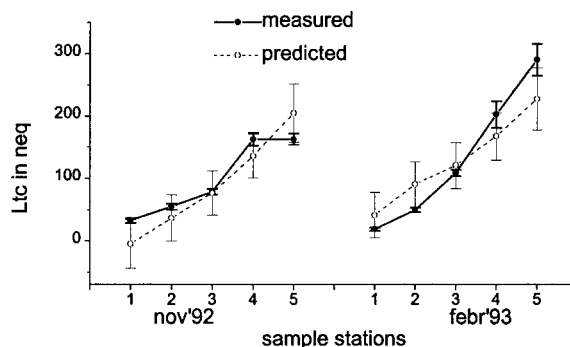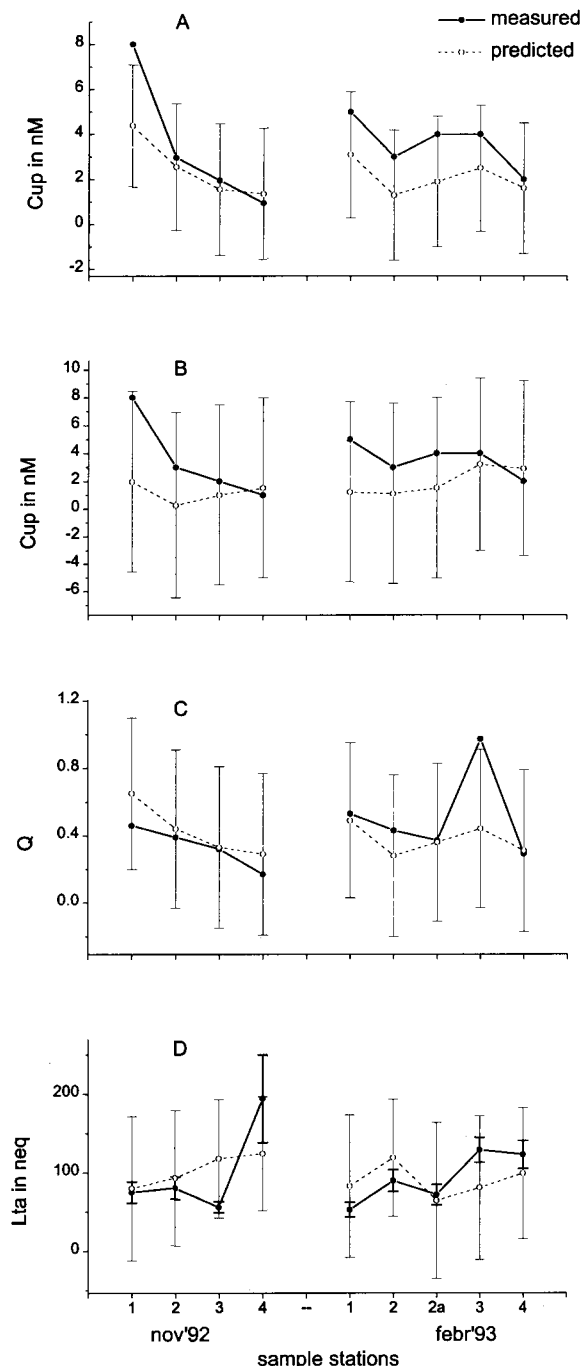

A

B

C

D

Fig. 2. Measured and predicted variables in the Oosterschelde using Eqs. 1, 2, 4 and 8 from Table 1. (A) Particulate Cu concentration (in nM) according to Eq. 1. (B) Particulate Cu concentration (in nM) according to Eq. 8. (C) The ratio Q of particulate Cu and dissolved Cu according to Eq. 2. (D) L_ta according to Eq. 4. The measured values are indicated by filled circles. In all figures, the 95% probability intervals of the predicted values are indicated, denoted by (thin) vertical lines. In (D), the 95% confidence intervals of the measurements are also indicated by (heavy) vertical lines, bracketing ligand concentrations estimated according to the Langmuir isotherm.

Fig. 3. Measured and predicted variables of ligand concentrations, L_tc, in the Westerschelde using Eq. 7 from Table 1. The measured values are indicated by filled circles. The 95% probability intervals of the predicted and measured values are indicated by vertical lines.

and dissolved Cu may also be assumed at those stations. From the measured $[Cu]_p$ and $[Cu]_d$, free Cu, and hence a good approximation of bio-available Cu, can be calculated for the Oosterschelde using the following equation (Gerringa et al., 1996a,b):

$$[Cu]_d/(Ka' \times L_ta + Kc' \times (L_tc - [Cu]_d))$$

$$= [Cu^{2+}] = [Cu]_p/(POC(\Gamma_{ads} \times B')) \qquad (9)$$

The left-hand side of Eq. 9 is valid when ionic Cu is assumed to be in equilibrium with the dissolved Cu phase ($[Cu]_d$). The right-hand side is valid when equilibrium is assumed to exist between ionic Cu and the particulate Cu phase ($[Cu]_p$). Here POC is in mol C and B' indicates the stability constant of adsorption of Cu onto POC and Γ_{ads} denotes the adsorption capacity for Cu per mol POC. From measurements, B' was estimated to be $10^{11.35}$ ($10^{11.22}$ to $10^{11.47}$ at 95% confidence limits), Γ_{ads} was estimated to be 0.01316 mol per mol POC (0.01122 to 0.01509 at

95% confidence limits). For the product $\Gamma_{ads} \times B'$ a mean value of $10^{9.47}$ will be used (Gerringa et al., 1996a,b).

Using the right-hand side of Eq. 9 and substituting the measured values of $[Cu]_p$ and POC values from the five stations in the Oosterschelde produced values of free Cu between 8 and 32 (± 20) fM $[Cu^{2+}]$ ($n = 16$). Using the binding characteristics of the ligands (error due to 95% confidence limits of K' and L_t in $[Cu^{2+}] = \pm 10$ fM per nM $[Cu]_d$) in the left-hand side of Eq. 9 resulted in $[Cu^{2+}]$ between 3 and 16 (± 50) fM ($n = 5$). Yet $[Cu^{2+}]$ at station 2 was 0.06 fM, but this was due to the large standard error of the measured characteristics of the strong ligand (Table 3).

The various estimates of $[Cu^{2+}]$ did not differ much. The comparable $[Cu^{2+}]$ values from the left- and right-hand side of Eq. 9 confirmed the assumption of equilibrium between particulate and dissolved Cu.

Valenta et al. (1986) measured salinity, POC and Cu fractions in the central part of the Oosterschelde, close to our stations 2 and 3, before the closure of the Oosterschelde (1979–1980). Applying Eq. 8 to the salinity and POC values of Valenta et al. (1986) resulted in particulate Cu concentrations half the value of the measured concentrations. This discrepancy is certainly due to the different conditions at that time, when the Oosterschelde was still a tidal sea arm with considerable freshwater input full of organic and inorganic pollutants (Wetsteyn and Bakker, 1991; Wetsteyn and Kromkamp, 1994). At that time, particulate and dissolved Cu were most probably not in equilibrium, just as in the Westerschelde,.

Since the mean adsorption capacity is 0.01316 mol Cu per mol POC, 1316 nmol Cu would be needed to saturate a concentration of 100 µM POC. This means that the Oosterschelde as it is now (without a salinity gradient, with low concentrations of suspended matter, with hardly a seasonal trend, no influence of fresh water, and hardly any pollution (Wetsteyn and Bakker, 1991)) is well buffered with respect to $[Cu^{2+}]$.

4.2. Westerschelde

In the Westerschelde, free Cu is predominantly influenced by a relatively strong dissolved organic ligand. This ligand is mainly supplied by the river Scheldt, but is partly of marine origin (Gerringa et al., 1996a,b) and its concentrations can be predicted with Eq. 7 if salinity and DOC are known. In comparison with Eq. 4 for L_ta in the Oosterschelde (Fig. 2D), Eq. 7 (Fig. 3) gives much more realistic 95% confidence intervals for L_tc in the Westerschelde.

Only equilibrium between ionic Cu and the dissolved ligands can be assumed in the Westerschelde. Since the concentrations of the two ligand groups are of the same order of magnitude and the Kc' is four orders of magnitude larger than Ka', only the characteristics of the relatively strong ligand determine ionic Cu. Introducing Eq. 7 into the left-hand side of Eq. 9 yields a $[Cu^{2+}] = 14$ (± 13) fM at station 2. The 95% confidence intervals are small (same order of magnitude as the concentration). Using the measured values for the weaker ligand in Eq. 9, no difference in free Cu was estimated; thus it was legitimate to neglect the term $K'a \times Lta$ in Eq. 9.

The combination of Eq. 7 (error due to the use of Eq. 7 0.2 fM per nM $[Cu]_d$) and Eq. 9 (error due to the 95% confidence interval of the strong ligand characteristics in $[Cu^{2+}] = 1.7$ fM per nM $[Cu]_d$) gave $[Cu^{2+}]$ between 7 and 35 (± 40) fM ($n = 16$), whereas using the measured ligand characteristics of the five stations in the left-hand side of Eq. 9 resulted in $[Cu^{2+}]$ between 1 and 16 (± 70) fM ($n = 10$). Due to the error in the ligand characteristics, the error in $[Cu^{2+}]$ is quite large.

Van den Berg et al. (1987) measured complexation along the Scheldt estuary. Their concentrations calculated from the measured ligand characteristics vary between 4 and 199 fM (mean 50 fM), without any obvious relation with the location of the sampling stations.

Considering the errors due to the measurement of the ligand characteristics, the free-Cu concentrations estimated from (1) the data in this study with Eq. 7 (7 to 35 fM), (2) the data in this study using the left-hand side of Eq. 9 (1 to 16 fM), and (3) the data in Van den Berg et al. (1987) (50 fM) are all in reasonable agreement with each other.

It is remarkable that the relationship describing L_tc as a function of DOC and salinity (Eq. 7) is applicable to other regions of the Westerschelde. It is unexpected that the prediction of ligand concentra-

tions, and thus of free-Cu concentrations, is so easy in a complex system such as this estuary. This is largely because Eq. 7 can only be used in that part of the estuary where no processes take place that have significant consequences for metal fractionation and speciation. At low salinities, in the maximum turbidity zone and in the region of oxidation most riverine particles have sedimented (Duinker et al., 1982; Regnier and Wollast, 1993; Zwolsman and Van Eck, 1993). In the salinity regions 10 to 25 or 30, where Eq. 7 can be used, Cu is released from particles due to oxidation and degradation processes taking place downstream of the region of oxidation. The dissolved Cu is complexed by dissolved organic ligands and thus kept in the dissolved phase (Regnier and Wollast, 1993). In the application zone of Eq. 7, the organic ligands are mostly riverine, but partly marine or estuarine. Where dilution and mineralisation reduce the riverine ligand concentrations too much, Eq. 7 is not valid any more. A comparison with previously reported dissolved-Cu concentrations (Zwolsman and Van Eck, 1993; Monteny et al., 1993; Van Alsenoy, 1993) suggests that not much happens in this more saline region of the estuary, which in turn explains why the results of the tidal-cycle experiment did not show a trend with salinity. This part of the Westerschelde is not as well buffered as the Oosterschelde, because the concentrations of the strong dissolved organic ligands are not much higher than the dissolved-Cu concentrations; an addition of 126 nM (8 ppb) might fill the strong ligand sites. When these ligand sites are full, the weaker ligands determine the free-Cu concentrations (left-hand side of Eq. 9), which will then rise to 30 pM.

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