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A review of total dissolved copper and its chemical speciation in San Francisco Bay, California

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Abstract

Following basin-wide contamination from industrial emissions and urban development, total dissolved copper concentrations in some regions of San Francisco Bay have exceeded national and state guidelines for water quality. In the face of dramatic improvements in wastewater treatment and point source control, persisting elevated dissolved copper concentrations in the Bay have prompted multiple studies and extensive monitoring of this estuary since 1989. Statistical analyses of monitoring data show that total dissolved copper concentrations have declined in the North (by 17%) and South (29%) San Francisco Bay as well as in the Southern Sloughs (44%) from 1993 to 2001. Concentrations remain elevated in the farthest reaches of the Bay (Delta and Estuary Interface), and in the Central Bay. Dissolved copper concentrations throughout the Bay have also been positively correlated ($r = 0.632$, $P < 0.0005$, $n = 598$) with dissolved organic matter, supporting results from complimentary chemical speciation studies which indicate that high-affinity copper-binding organic ligands dominate the chemical speciation of dissolved copper in the Bay. These organic ligands typically bind $> 99.9\%$ of the dissolved copper, effectively buffering the system against small changes in dissolved copper concentrations, and maintaining free Cu^{2+} concentrations well below the toxicity threshold of ambient aquatic microorganisms. In response to these findings, site-specific water quality criteria for dissolved copper concentrations are now being developed by the Regional Water Quality Board to provide a more appropriate standard for copper toxicity in the Bay—one based on its chemical speciation and bioavailability.
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1. Introduction

Dissolved copper plays the role of both a micronutrient (required for optimal growth) and a toxicant (impeding growth) to phytoplankton and other microorganisms in natural waters (e.g., Manahan and Smith, 1973; Brand et al., 1986; Peers et al., 2005). In San Francisco Bay, elevated dissolved copper concentrations have generated concern for copper toxicity in this system as total dissolved copper concentrations approach and sometimes exceed state and national water quality guidelines. Copper toxicity, however, is proportional to the concentration of *inorganic* (Cu^{2+}, Cu') copper species, rather than the sum of all copper species (including inorganic and organically complexed, and true ionic species) that comprise a *total dissolved* ($< 0.4 \, \mu m$ filter pore size) copper measurement.

The free hydrated Cu^{2+} ion acts as a toxicant to phytoplankton by antagonizing uptake sites for the essential micronutrients Mn^{2+} and Zn^{2+} (Sunda and Huntsman, 1983, 1998). For example, at elevated $[Cu^{2+}]$, the Cu^{2+} ions compete for Mn^{2+} uptake sites, inhibiting adequate uptake of Mn^{2+}, a micronutrient required for achieving optimal growth. At the higher concentrations of Cu^{2+} ($\sim 10^{-11} \, M$) that can exist in estuarine environments, copper will become toxic to aquatic microorganisms (Brand et al., 1986; Sunda et al., 1987).

San Francisco Bay has been subjected to relatively high contaminant metal inputs since the 1848 Gold Rush, a problem compounded by the subsequent urbanization of

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the estuary and its watershed over the past one and a half centuries. Elevated concentrations of some metal contaminants, including copper, have persisted in the face of technological advancements in water treatment and increased regulation, prompting far-reaching investigations into the bioavailability (and corresponding toxicity) of metal contaminants and water quality monitoring in the Bay. The San Francisco Bay Regional Water Quality Control Board (Regional Board) implemented, through permit conditions, the San Francisco Estuary Institute's Regional Monitoring Program for Trace Substances (RMP) in 1993 (Hoenicke et al., 2003). This program has provided a systematic collection of *total dissolvable* (unfiltered acidified) and *total dissolved* ([Cu_T], < 0.45 µm filter pore size acidified) metal concentrations, including copper, from 26 spatially distributed stations along the central axis of the Bay for over a decade (Sañudo-Wilhelmy et al., 2004).

Collections for the RMP over the first decade were designed to sample the Bay during three different hydrologic regimes (Flegal et al., 1991). These regimes correspond with the seasonality of freshwater discharges to the estuary: during the winter (December–March), when the bulk of annual precipitation in the watershed occurs and freshwater discharges are highest; spring (April–May), when precipitation is lower and most fresh water discharges are due to snow melt from the Sierra Nevada mountains; and the late summer (July–August), when precipitation is typically negligible and a minimal level of fresh water runoff is sustained by water management practice. Although the temporal sampling design of the RMP revealed seasonal differences in dissolved copper concentrations, as well as those of other trace elements, marked intra-annual and inter-annual variations in freshwater discharges complicated assessments of systematic temporal changes in those concentrations, and RMP collections as of 2002 were correspondingly isolated to dry period (late summer) sampling (Flegal et al., 2005).

Coincident with the monitoring of spatial and temporal variations in total dissolvable and total dissolved copper concentrations, were more detailed studies on the biogeochemical cycling of copper within the Bay (Donat et al., 1994; Sañudo-Wilhelmy et al., 1996; Rivera-Duarte and Flegal, 1997; Sedlak et al., 1997; Luoma et al., 1998; Duke et al., 1999; Lessin, 2001; Ostrowski, 2001; Beck et al., 2002; Gee and Bruland, 2002; Buck and Bruland, 2005; Hurst and Bruland, 2005; Ndung'u et al., 2005). Of significance, chemical speciation studies were catalyzed by research showing that as much as 99% of the total dissolved copper in adjacent northeast Pacific coastal waters was tightly bound to organic ligands and, therefore, not readily available as either a nutrient or toxicant to phytoplankton in those waters (Coale and Bruland, 1988). Those determinations were especially relevant for San Francisco Bay, where copper concentrations can exceed state and federal water quality criteria—criteria based on *total dissolved* concentrations.

As was determined by Coale and Bruland (1988) in the NE Pacific, chemical speciation studies have indicated that concentrations of the inorganic (potentially toxic) copper species in San Francisco Bay are not consistently related to total dissolved copper concentrations (Donat et al., 1994; Beck et al., 2002; Buck and Bruland, 2005; Hurst and Bruland, 2005). Recent work has reinforced these observations, finding that > 99.9% of the dissolved copper in the Bay exists as organic (CuL_i) copper species (Buck and Bruland, 2005; Hurst and Bruland, 2005). As such, total dissolved copper concentrations are not diagnostic of toxicity in this system, and the distribution of copper between the different chemical species must be taken into consideration in establishing more appropriate water quality standards for dissolved copper.

To determine the toxicity of total dissolved copper concentrations in the Bay, and the appropriate copper criteria to protect this ecosystem, the quantification and cycling of organic copper complexation has become increasingly important. Accordingly, the following review is composed of two primary sections. The first summarizes studies on spatial and temporal variations in *total dissolved* copper concentrations in the Bay, providing a first-order measure of recent trends in copper contamination in the estuary. The second summarizes complementary studies on *copper speciation* in the Bay, illustrating the importance of organic complexation of copper on its bioavailability and toxicity.

2. Total dissolved copper

2.1. Historical point sources

With the California Gold Rush of 1848, people flocked to the San Francisco Bay area, transforming the trading post of 400 into a bustling hub of 25,000 within a 2-year period (Nichols et al., 1986). The progressive development and urbanization of this estuary over the last century and a half has had extensive impacts on the water quality of the Bay: from changes in flow and bathymetry that alter the residence time and flushing characteristics of the Bay, to elevated metal contaminant inputs and potential toxicity events.

Anthropogenic impacts on water quality in San Francisco Bay, dubbed the 'Urban Estuary' (Nichols et al., 1986), has been a key interest of both policymakers and scientists over the past several decades. A timeline of the major progressions in our scientific understanding of copper cycling and toxicity in San Francisco Bay, as well as the policy changes that have incorporated this understanding to alter regulatory approaches to water quality and copper toxicity in the Bay, are presented in Fig. 1.

Increased sedimentation from early mining activities resulted in fundamental bathymetric changes, reducing tidal circulation, as well as water volume capacity—ultimately leading to the current hydrography of the Bay (Nichols et al., 1986). Modern hydrography naturally
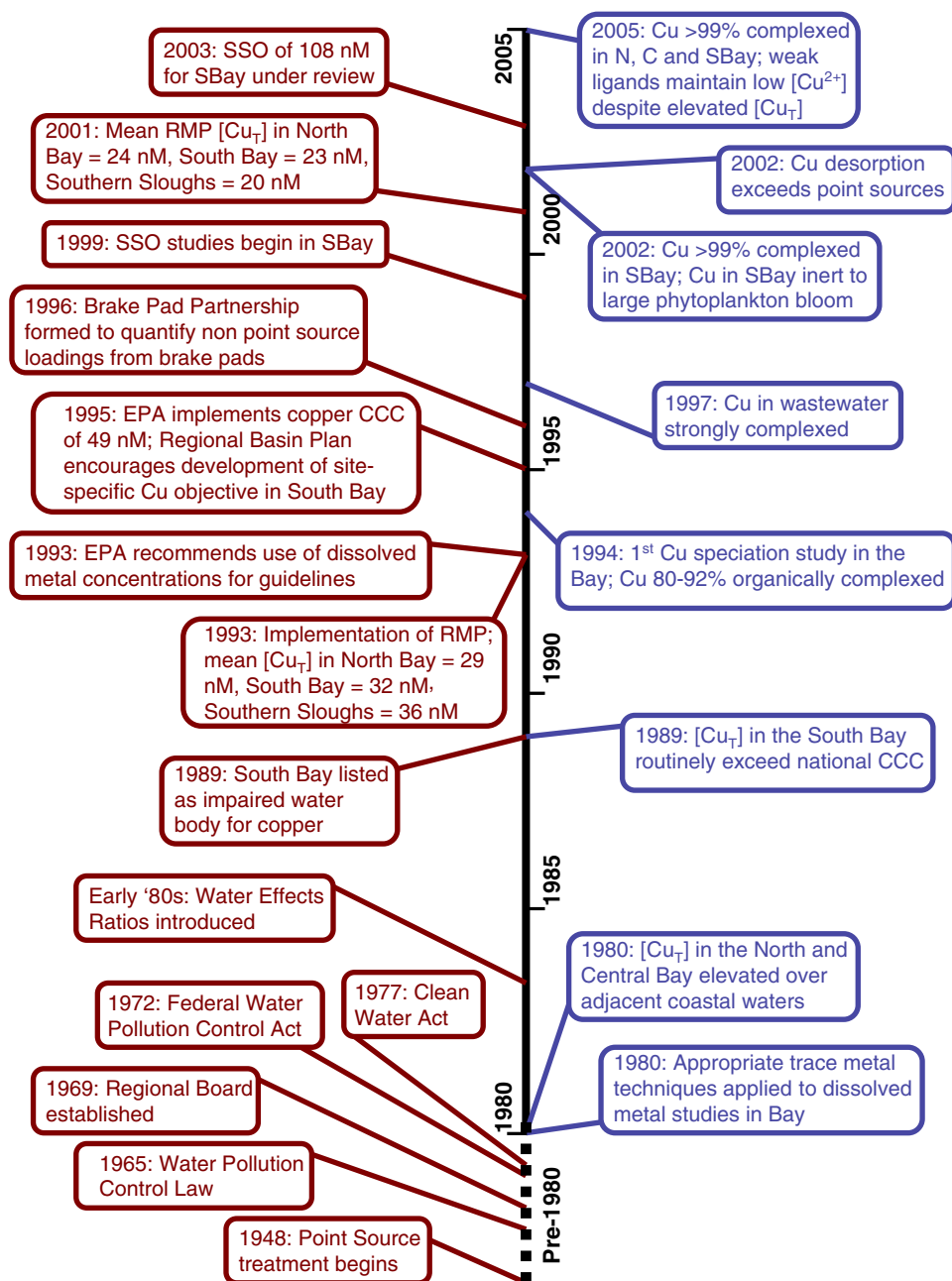
Fig. 1. Timeline of key scientific advancements in copper research in San Francisco Bay, CA over the last several decades and the corresponding policy developments.

divides San Francisco Bay into three principal regions: North Bay (including San Pablo Bay, Suisun Bay, and the Sacramento River/San Joaquin River Delta), Central Bay, and South Bay. Each of these regions can be further subdivided such that the Delta, where the San Joaquin and Sacramento Rivers merge into the estuary, can be distinguished from the rest of the North Bay. Similarly, in the extreme reaches of the South Bay, the Estuary Interface and the Southern Sloughs can be considered distinct regions—as they are in subsequent statistical analyses (Fig. 2). The differentiating geographic and hydrographic characteristics of these regions contribute

to marked spatial variations in dissolved copper concentrations and trends (Flegal et al., 1991, 2005).

Although the first quantitative loadings measurements were not undertaken until the late 1970s (Luoma and Phillips, 1988), dated sediment core analyses have shown that the urbanization of San Francisco Bay over the past 150 years has been accompanied by elevated metal loadings (Hornberger et al., 1999). Increased metal concentrations resulting from these anthropogenic inputs have proved persistent, raising concern for copper toxicity events and the resulting complications to the local ecosystem (Nichols et al., 1986; Kuwabara et al., 1989; Flegal et al., 2005).
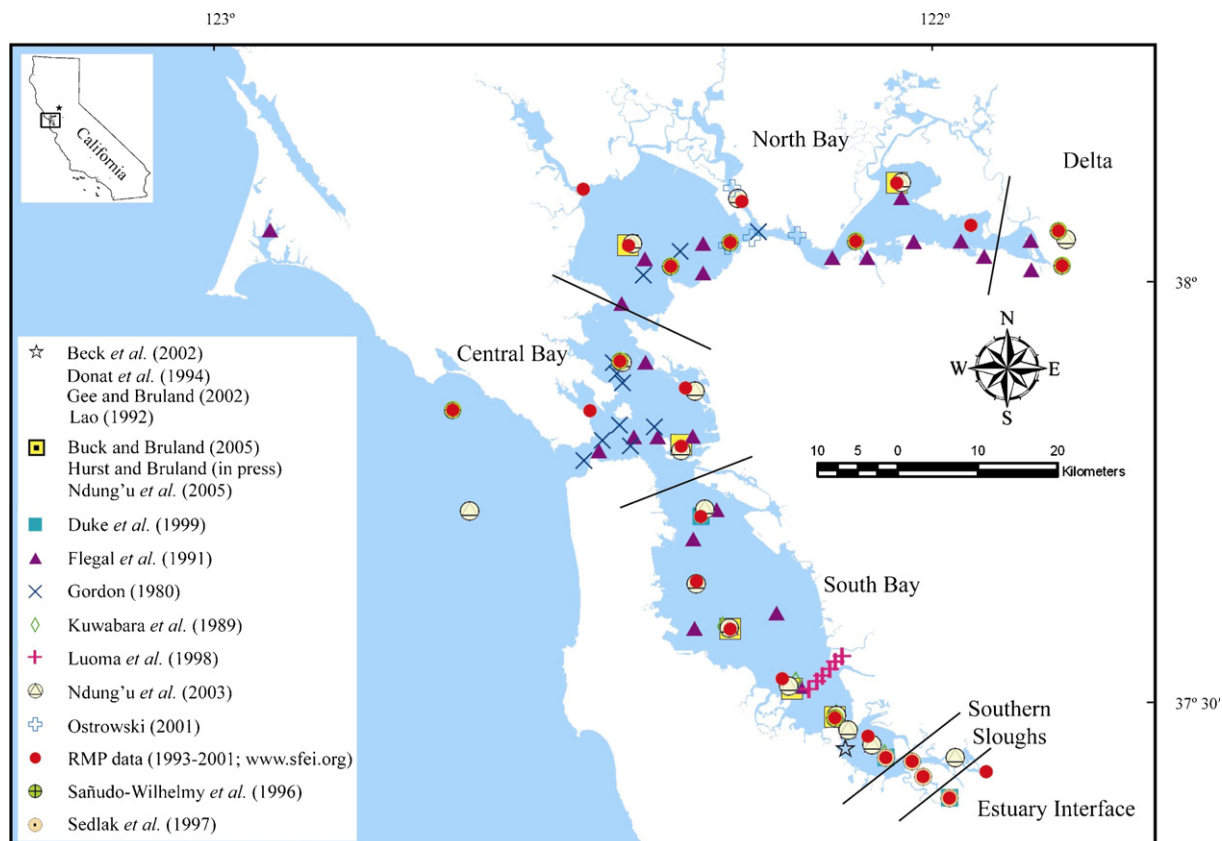
Fig. 2. Sampling sites from dissolved copper studies in San Francisco Bay, CA beginning with Gordon (1980). The six regions of the Bay, as determined by distinguishing hydrology, geography and water chemistry, are also indicated.

Recognizing the potential impact of these high metal concentrations, state and federal agencies have striven to reduce pollutant loadings to the Bay, spending billions of dollars to improve water quality (Flegal et al., 2005).

While original point source water treatment was initiated in 1948, the procedure was substantially overhauled with the Water Pollution Control Law of 1965 to provide the necessary reductions in metal loadings (State Water Resources Control Board, 1969). In 1975, copper loadings from some of the major publicly operated treatment works in the South Bay were at 343 kg Cu/d. By 1985, loadings from the same treatment works had declined to 52 kg Cu/d, a reduction of nearly 85% (Davis et al., 1991; Flegal et al., 1991). Loadings have continued to decline since 1985, and a subsequent report (Davis et al., 1991) estimated that copper loadings in wastewater discharge were 28–46 kg Cu/d in the South Bay and even less in the Central Bay (8–13 kg Cu/d) and Delta (7–9 kg Cu/d). South Bay treatment plants now effectively remove an average of 90% of the copper from influent wastewaters, further lowering wastewater copper loadings (Tetra Tech Inc. et al., 2000; City of Palo Alto Regional Water Quality Control Plant, 2003).

US Environmental Protection Agency (EPA) water quality guidelines for copper were initially written as *total dissolvable* (unfiltered acidified samples) copper concentrations, and the bioavailability of this copper was calculated

from an empirical relationship between copper toxicity and water hardness—a relationship primarily of importance in freshwater environments (US EPA, 2003). Beginning in the early 1980s, the EPA began incorporating Water Effects Ratios (WER) into their guideline calculations. The WER was introduced to account for additional water quality parameters beyond hardness, by reflecting the difference in toxicity guideline outcomes between ambient waters and laboratory waters adjusted to the same water hardness values. The incorporation of WER has greatly enhanced water quality guidelines by taking the ambient water chemistry of a site into account when determining the relationship between metal measurements and toxicity.

In 1993, the EPA switched water quality guidelines from total dissolvable to *total dissolved* (acidified, filtered—$0.45 \, \mu m$ pore size) copper concentrations, in recognition of studies showing that copper bioavailability is associated with the dissolved Cu^{2+} ion species (US EPA, 1995). Chronic dissolved criteria were implemented in 1995, stipulating a national criterion continuous concentration (CCC) of 49 nM (3.1 µg/L) for dissolved copper in US coastal waters (US EPA, 1995). However, studies in San Francisco Bay have indicated that copper cycling in this estuarine environment is different from that in coastal waters, with extensive organic complexation of copper persisting throughout the Bay. Currently, a site-specific water quality objective (SSO) for dissolved copper is being

developed in South San Francisco Bay, using an appropriate WER to incorporate the ambient chemical speciation observed (City of San Jose Environmental Services Department, 1998).

2.2. Historical studies of copper distributions

Although total dissolved copper concentrations are relatively high (up to 93 nM) in San Francisco Bay, appropriate precautions must be taken in both analysis and sampling processes to prevent contamination in measurements at the nanomolar (nM = 10^{-9} mol/L) level (Bruland et al., 1979). Therefore, while there are several reports of dissolved copper concentrations in the Bay prior to 1980, most of those measurements did not utilize rigorous trace metal clean techniques and are considered questionable (Flegal et al., 1991). For the purposes of this review, only data employing suitable techniques will be presented. All sampling stations in the San Francisco Bay copper studies, beginning in 1980, are presented in Fig. 2. Table 1 lists the distribution of these stations among the six main regions of the Bay, and the years these regions were sampled for both independent and monitoring studies.

The first study to incorporate appropriate techniques (Gordon, 1980) measured total dissolved copper concentrations at 11 sites in the North and Central Bay and 6 sites in adjacent coastal waters. With sites in the North and Central Bay, Gordon (1980) found total dissolved copper concentrations in the Bay (6.5–43.4 nM) to be substantially higher than coastal water concentrations (1.1–6.8 nM) outside the Bay. North and Central Bay concentrations generally followed conservative mixing lines (e.g., mixing lines between high [Cu_T], low salinity Delta water and low [Cu_T], high salinity Golden Gate seawater) under higher (930 m^3/s in 1979, 2160 m^3/s in 1980) Delta outflow conditions (Gordon, 1980). However, during low Delta outflow (~70 m^3/s in 1979) dissolved copper concentrations at high salinity values (9–16 nM at $S = 31–33$) in the North and Central Bay were elevated over similarly high salinity values (6.5–8 nM at $S = 28–30$) during the higher Delta outflow periods (Gordon, 1980). Although this study did not include any South Bay stations, Gordon (1980) suggested that the elevated copper concentrations observed at high salinity values may have been due to anthropogenically contaminated high salinity South Bay waters mixing into Central Bay waters under low Delta outflow conditions.

From these observations, much of the interest on metal contamination levels shifted to the South Bay, with several subsequent studies focused on this particular region. In 1985, Kuwabara et al. (1989) sampled four stations in the South Bay at five sampling intervals in the spring and late summer. Total dissolved copper concentrations in the South Bay were found to routinely exceed the national CCC guideline of 49 nM, and these concentrations, which ranged from 29 to 66 nM over all sampling periods, did not

Table 1
Sampling regions, stations, years, and corresponding authors of total dissolved copper studies in San Francisco Bay, CA from Gordon (1980) to Buck and Bruland (2005)

Region	# Stns	Yrs sampled	Authors
Delta	1	1989	Flegal et al. (1991)
	1	1993, 1995	Ndung'u et al. (2003)
	2	1993–2001	RMP/WIGS (www.sfei.org)
	2	1994	Sañudo-Wilhelmy et al. (1996)
North Bay	6	1979–1980	Gordon (1980)
	14	1989	Flegal et al. (1991)
	8	1993–2001	RMP/WIGS (www.sfei.org)
	3	1993, 1995	Ndung'u et al. (2003)
	4	1994	Sañudo-Wilhelmy et al. (1996)
	4	1999	Ostrowski (2001)
	2	2003	Buck and Bruland (2005)
	1	2003	Hurst and Bruland (2005)
	2	2003	Ndung'u et al. (2005)
Central Bay	5	1979–1980	Gordon (1980)
	5	1989	Flegal et al. (1991)
	6	1993–2001	RMP/WIGS (www.sfei.org)
	4	1993, 1995	Ndung'u et al. (2003)
	1	1994	Sañudo-Wilhelmy et al. (1996)
	1	2003	Buck and Bruland (2005)
	1	2003	Hurst and Bruland (2005)
	1	2003	Ndung'u et al. (2005)
South Bay	4	1985	Kuwabara et al. (1989)
	7	1989	Flegal et al. (1991)
	1	1991	Donat et al. (1994)
	1	1992	Lao (1992)
	3	1992, 1994	Duke et al. (1999)
	6	1993–2001	RMP/WIGS (www.sfei.org)
	7	1993, 1995	Ndung'u et al. (2003)
	6	1994	Luoma et al. (1998)
	1	1994	Sañudo-Wilhelmy et al. (1996)
	1	1996, 1999–2000	Beck et al. (2002)
	1	1996–1997	Sedlak et al. (1997)
	1	1997	Gee and Bruland (2002)
	3	2003	Buck and Bruland (2005)
	3	2003	Hurst and Bruland (2005)
	3	2003	Ndung'u et al. (2005)
Southern sloughs	2	1996–2001	RMP/WIGS (www.sfei.org)
	1	1993, 1995	Ndung'u et al. (2003)
	3	1996–1997	Sedlak et al. (1997)
Estuary interface	1	1992, 1994	Duke et al. (1999)
	2	1994–2001	RMP/WIGS (www.sfei.org)
	1	1996–1997	Sedlak et al. (1997)

follow any resolvable spatial or temporal patterns. Instead, dissolved copper concentrations over both sampling periods were determined to be positively correlated ($r^2 = 0.45$, simple linear regression) with dissolved organic carbon (DOC), suggesting that distributions of dissolved copper in the South Bay are likely controlled by complexation with ambient dissolved organic matter.

The complexity of results from early studies provided much of the rationale for a more resolved, both temporally and spatially, investigation into trace metal cycling in the Bay. The Regional Board initiated such an investigation in 1989 (Flegal et al., 1991), with collections made in April (spring), August (late summer) and December (winter) of 1989, including samples for measurements of total dissolvable (unfiltered acidified) and total dissolved (< 0.45 μm filter pore size, acidified) metal (including copper) concentrations. Samples were obtained along the central channel of the Bay such that multiple stations were sampled in each of the six hydrographic regions (Fig. 2). Total dissolved concentrations of copper at comparable salinity values in the South Bay (32–52 nM at $S = 31$) were found to exceed concentrations in the North Bay (13–17 nM at $S = 30-31$) over all three sampling periods. Copper excesses beyond conservative mixing lines were tentatively attributed to anthropogenic inputs—including wastewater discharges, urban runoff, and the diagenetic remobilization of copper from historically contaminated sediments.

This 1989 study (Flegal et al., 1991) served as the predecessor for the implementation of the San Francisco Bay Regional Monitoring Program for Trace Substances (RMP) in 1993. With the realization of extensive monitoring of total dissolved copper concentrations, copper research in the Bay moved on to the biogeochemical cycling and bioavailability of copper. The ongoing RMP monitoring has been complemented by studies of the distribution processes, fluxes, and chemical speciation of dissolved copper in the Bay. Due to the inherent dependence of copper bioavailability on dissolved copper speciation, much research has focused on the complexation of dissolved copper with strong copper-binding organic ligands; the results from these studies are further discussed in the Dissolved Copper Speciation section to follow.

2.3. Diagenetic remobilization and phase partitioning

In their review of dissolved metal contaminant concentrations sampled through 1989 in San Francisco Bay, Flegal et al. (1996) stressed the potential importance of the diagenetic remobilization of these metals back into the dissolved phase from suspended sediments. The authors predicted that fluxes of dissolved metals from sediments would become an increasingly important internal source, especially as other sources, including point source and freshwater inputs, declined with improved treatments and continued diversion of flow from the Delta region. The long hydraulic residence times observed in the South Bay (~120 days during high discharge and ⩾160 days under low discharge) further implicated this particular region as a hot spot for sediment–water column interactions (Flegal et al., 1991).

While the *diffusive* flux of copper from the sediments is seasonally variable, alternately acting as a source and as a sink for dissolved copper throughout the Bay

(Rivera-Duarte and Flegal, 1997; Spinelli et al., 2002), Gee and Bruland (2002) determined that remobilization of copper by *desorption* from resuspended sediment particles within the water column is a much larger flux. From their work, Gee and Bruland (2002) estimate that desorption of copper from resuspended sediments exceeds combined point source dischargers as a primary copper source in the South Bay. Consequently, much of the elevated concentrations of dissolved copper in the Bay is currently attributed to its partitioning from contaminated sediments in the Bay.

Copper partitioning is defined by the concentration of copper in the dissolved phase versus the concentration of copper available from the particulate phase (Gee and Bruland, 2002). The partitioning of copper between dissolved and particulate phases in the San Francisco Bay has been found to be highly variable, and this variability has proven difficult to explain (Gordon, 1980; Luoma and Phillips, 1988; Kuwabara et al., 1989; Sañudo-Wilhelmy et al., 1996; Luoma et al., 1998; Gee and Bruland, 2002). At least some of the variability in copper partitioning measurements is likely related to the different techniques used to measure these phases.

The concentration of copper in the dissolved phase, the *total dissolved* copper concentration ($[Cu_T]$), is measured in filtered and acidified samples. There are two common pore sizes used in these measurements: 0.2 and 0.45 μm. Both of these pore sizes exclude the particulate size class, but different portions of the colloidal size fraction may be included or excluded depending on the pore size. As less than 10% of dissolved copper in the Bay exists in the colloidal size-fraction, the difference in filter pore sizes used (0.2–0.45 μm pore size) for dissolved copper measurements should not be a relatively large source of variability in the measurements of copper partitioning (Sañudo-Wilhelmy et al., 1996).

The particulate phase of copper is quantified in terms of the *exchangeable particulate* copper. The exchangeable particulate copper measured is intended to indicate the amount of copper associated with suspended sediments that has the potential to exchange into the dissolved phase. This is quantified as the copper that can be leached from filtered particulates with a weak acid solution over a defined time period. The exchangeable particulate copper can also be quantified as the total dissolvable copper concentration (acidified unfiltered concentration) minus the total dissolved concentration (acidified filtered—0.45 μm). In either approach, particles are not completely digested, and the techniques are, thus, sensitive to changes in acid strength and exposure time. These sensitivities, and the discrepancies between methods used, may explain some of the variability observed in copper partitioning calculations.

The partitioning of copper between dissolved and particulate phases is additionally influenced to some undefined extent by organic complexation. Numerical modeling (ELAmet) of existing dissolved and exchangeable particulate copper data found that increasing the

complexation of dissolved copper (by increasing organic matter content) in the model further drove copper into the dissolved phase, making it less available to the particulate phase (Wood et al., 1995). Further, Wood et al. (1995) suggested that discrepancies in observed distributions of copper between the dissolved and particulate phases was likely due to the kinetics of partitioning and complexation, which are not well understood. The variable nature of copper partitioning in the Bay, and the importance of organic complexation and partitioning kinetics in constraining this copper have also been recognized in a Conceptual Model Report sponsored by the City of San Jose and the Regional Board (Tetra Tech Inc., 1999).

To address the kinetics of copper partitioning in the South Bay, Gee and Bruland (2002) followed the equilibration times of unfiltered South Bay waters spiked with a low abundance stable copper isotope (^{65}Cu). The kinetics of partitioning was fast (most of the spike adsorbed to the particulate phase within 20 min) when dissolved copper concentrations after the spike were high enough to potentially exceed the organic copper-binding ligand concentrations. Partitioning kinetics were much slower (estimated equilibration time ~3 weeks) when the dissolved copper additions were equilibrated with the dissolved phase

(and the copper-binding ligands) before spiking. These results indicate that the evaluation of partitioning kinetics is complicated by the strong complexation of dissolved copper in the natural waters of the South Bay. In the absence of kinetic data, and a better understanding of the quantitative relationship between organic complexation and copper partitioning, it is difficult to interpret trends in the exchangeable particulate data. These data have accordingly been excluded from statistical analyses, as further work in constraining the kinetics and ambient partitioning of copper in the Bay is required.

2.4. RMP spatial distributions

To best control uncertainty, the 1993–2001 RMP dissolved copper data and related measurements (e.g., DOC) generated by the RMP were used exclusively for all statistical analyses (Fig. 3). These data have been collected at the same sample sites and analyzed using extensively inter-calibrated methods (Flegal et al., 1991, 2005; Ndung'u et al., 2003), which have enabled statistical comparisons of temporal variations in other elemental concentrations in the Bay (Squire et al., 2002). For the following statistical analyses of dissolved copper spatial distributions, RMP
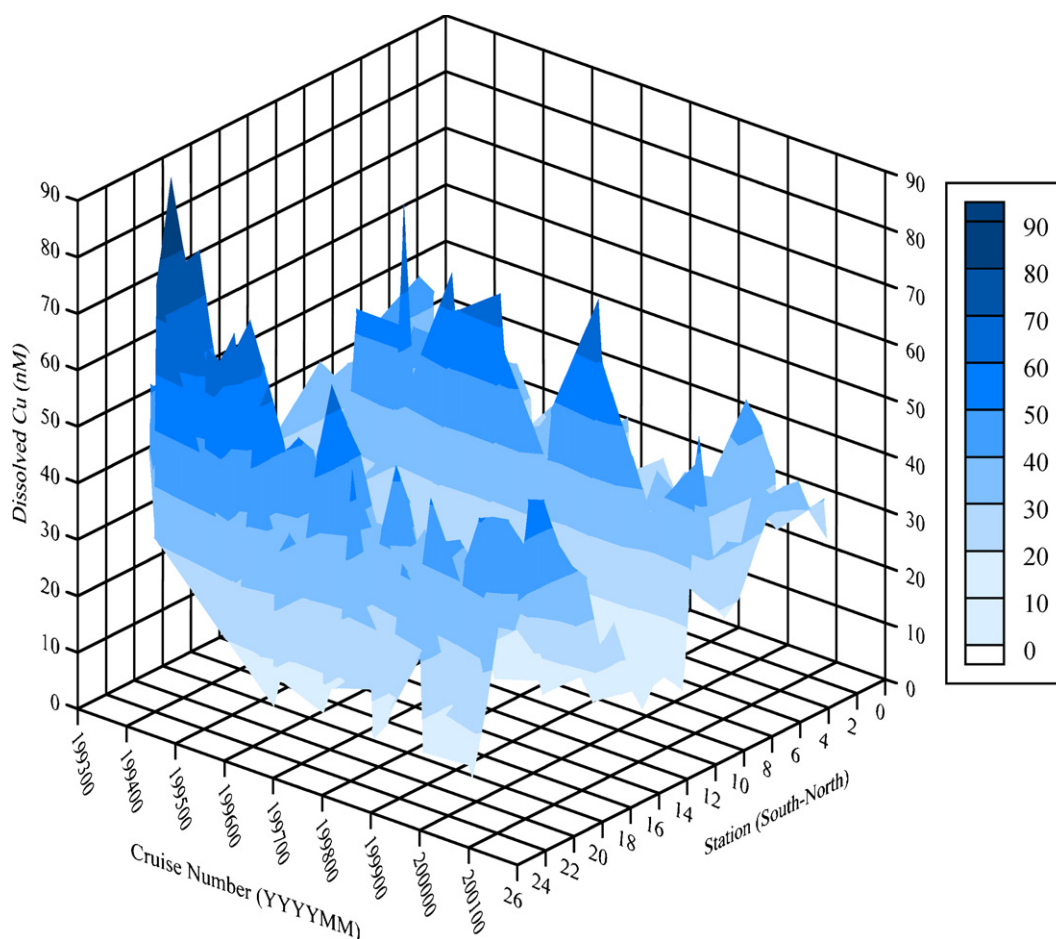

Fig. 3. Total dissolved copper concentrations from Regional Monitoring Program for San Francisco Bay, CA. Three-dimensional figure depicted as a function of time from 1993 to 2001 and over the space of the 26 stations monitored in the estuary by this program (www.sfei.org).

sampling stations were grouped by hydrographic region: the Estuary Interface (two stations), Southern Sloughs (two stations), South Bay (seven stations), Central Bay (five stations), North Bay (eight stations), and Delta (two stations) (San Francisco Estuary Institute, 2001).

Comparisons between these estuary regions, as well as sampling seasons and individual sampling stations, were conducted using the non-parametric Kruskal–Wallis test for multiple comparisons (Zar, 1984). Median and inter-quartile range (IQR) values for stations, regions, and seasons are presented in Table 2. If the null hypothesis, stating that the sample distributions were from the same population, was rejected ($P < 0.05$), then a non-parametric multiple comparison for unequal sample sizes was

Table 2
Median [Cu_T] and interquartile range (IQR) values resulting from individual station, region, and season comparisons of Regional Monitoring Program data in San Francisco Bay, CA, 1993-2001 (www.sfei.org)

Station	Median (nM)	IQR (nM)	n
Sacramento River	23.9	8.4	25
San Joaquin River	26.9	9.4	25
Honker Bay	27.4	10.3	22
Grizzly Bay	29.8	9.5	25
Pacheco Creek	29.7	9.3	25
Napa River	28.8	7.3	25
Davis Point	25.7	7.1	25
Pinole Point	23.8	6.4	25
San Pablo Bay	25.3	6.8	25
Petaluma River	51.9	20.9	22
Red Rock	17.3	10.8	22
Point Isabel	17.8	4.2	25
Richardson Bay	15.9	6.8	25
Golden Gate	6.8	3.5	22
Yerba Buena Island	18.6	4.8	25
Alameda	23.1	10.5	22
Oyster Point	22.8	11.5	25
San Bruno Shoal	29.0	10.1	22
Redwood Creek	33.8	7.7	25
Dumbarton Bridge	40.8	13.8	25
South Bay	45.5	16.0	25
Coyote Creek	48.3	26.1	22
San Jose	39.1	22.4	22
Sunnyvale	36.3	27.4	22
Standish Dam	21.8	10.4	16
Guadalupe River	14.8	8.9	13
Region			
Delta	26.1	7.6	50
North Bay	28.6	10.3	194
Central Bay	16.4	7.6	119
South Bay	32.0	19.0	166
Southern Sloughs	36.8	25.1	44
Estuary Interface	20.6	11.3	29
Season			
Winter	27.9	12.4	216
Spring	24.5	17.4	167
Late summer	26.7	16.1	219

performed in a manner paralleling the Tukey test, with mean ranks being used instead of means (Zar, 1984).

Significant ($H = 237.30$, df = 5, $P < 0.0005$) differences were found in [Cu_T] among the six regions of San Francisco Bay. Ranking by median were the Southern Sloughs (37 nM) > South Bay (32 nM) > North Bay (29 nM) > Delta (27 nM) > Estuary Interface (21 nM) > Central Bay (16 nM). Non-parametric multiple comparisons show the Southern Sloughs, where wastewater conveyances discharge, were significantly ($P < 0.05$) higher in [Cu_T] than all regions other than the South Bay. The Central Bay, at the mouth to the Pacific Ocean, was significantly ($P < 0.05$) lower in dissolved copper than all regions excluding the Estuary Interface.

Significant ($H = 367.33$, df = 25, $P < 0.0005$) differences in [Cu_T] were also found among the individual stations. Petaluma River in the North Bay was found to be significantly ($P < 0.05$) higher in dissolved copper (median [Cu_T] ≈ 52 nM) than the other stations, with the exception of four stations in the South Bay, one other North Bay station, and both Southern Sloughs stations. No significant ($H = 1.58$, df = 2, $P = 0.454$) seasonal differences were found between the three sampling periods, and only one South Bay station (San Lorenzo Creek) presented significant ($P < 0.05$) differences in [Cu_T] over seasonal time scales (Duke et al., 1999). This lack of significant seasonal trends in the RMP data corroborates earlier observations of dissolved metal loadings from urban point discharges in the South Bay and Estuary Interface regions (Duke et al., 1999).

2.5. RMP temporal trends

The influence of DOC, salinity, and daily Delta outflow (calculated by the California Department of Water Resources, see http://iep.water.ca.gov/dayflow/output/index.html), on [Cu_T] was examined using a best subset regression analysis. Dissolved copper, DOC, and daily Delta outflow measurements were $\log(X+1)$ transformed in an attempt to normalize the data. The results indicate that the best model (i.e., one with highest adjusted r^2 taking standard deviation, Mallows Cp, collinearity, and number of model predictors into account) was the one of dissolved copper concentrations regressed against DOC and Delta outflow. A significant (adj-$r^2 = 0.400$, $P < 0.0005$, $n = 598$) positive relationship was observed between dissolved copper and DOC (Fig. 4), with [Cu_T] expected to increase at increasing DOC concentrations. After removing the influence of DOC, a significant (adj-$r^2 = 0.177$, $P < 0.0005$, $n = 598$) negative relationship was documented between dissolved copper and daily Delta outflow.

Dissolved copper was found to have a significant but poor (adj-$r^2 = 0.029$, $P < 0.0005$, $n = 600$) negative relationship with salinity: the addition of salinity to the model increased explanatory power by less than 1% and also resulted in an increased collinearity among predictors. Salinity was, therefore, excluded as a predictor from the
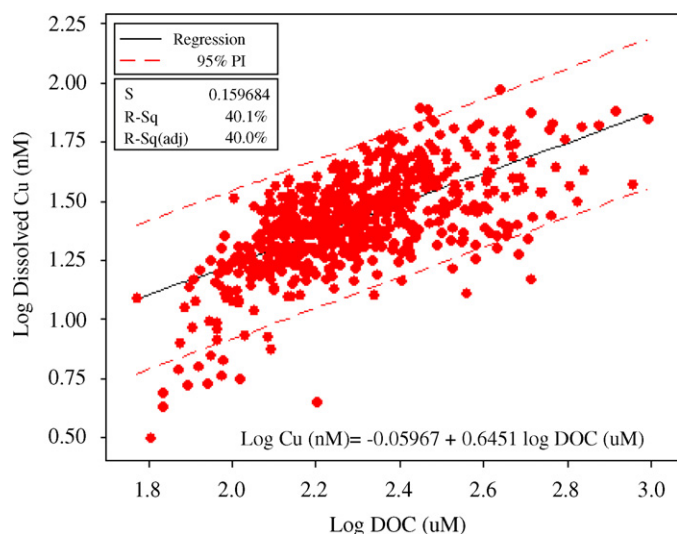
Fig. 4. Relationship between DOC and total dissolved copper concentrations from Regional Monitoring Program data set.

model. This result suggests dissolved copper concentrations measured over the last decade may not be as strongly correlated with salinity as observed in preliminary studies in the late 1970s and 1980s (Gordon, 1980; Luoma and Phillips, 1988; Kuwabara et al., 1989). Rather, the majority of variance in $[Cu_T]$ reported over this monitoring period is explained by changes in DOC and daily Delta outflow conditions, with this combination explaining (*adj-r^2* = 0.528, *P* < 0.0005, *n* = 595) ~53% of the variation in the data.

An analysis of covariance (ANCOVA) showed a significant difference among the slopes of the regression lines for each individual sampling station for both log-transformed DOC ($F_{25,546}$ = 1.55, *P* = 0.043) and log-transformed Delta outflow ($F_{25,550}$ = 3.33, *P* < 0.0005). Therefore, the individual station regression lines, instead of a common regression slope, were used to normalize the data (Hebert and Keenleyside, 1995). Log-transformed $[Cu_T]$ at each station were normalized for log-transformed daily Delta outflow and log-transformed DOC using multiple linear regression analysis. Daily Delta outflow and DOC were the independent variables and $[Cu_T]$ the dependent variable. Residuals from this analysis represent the variation in dissolved copper concentration that remains after the influence of DOC and daily Delta outflow have been removed, allowing for the quantification of temporal trends in $[Cu_T]$.

These temporal trends were then determined for each station by performing a linear regression analysis using the residuals from the multiple regression analysis as the dependent variable and sampling date as the independent variable. Combining individual station residuals permitted the examination of temporal trends by region. The presence of first-order autocorrelation was investigated, as appropriate, using the Durbin–Watson test, and corrected using the Hildreth–Lu procedure. A significant

positive (or negative) slope (*P* < 0.05) indicates an increase (or decrease) in dissolved copper concentrations at the station over time, while a lack of significance indicates no detectable change in dissolved copper concentration.

Temporal changes in total dissolved copper concentrations were evaluated at two levels: as individual stations (Table 3), and as regions of multiple stations (Table 4). Significant (see Table 3) decreases in dissolved copper concentrations from 1993 to 2001 were observed in data from all South Bay stations, one Central Bay station (Yerba Buena Island), one station (San Jose) in the Southern Sloughs and two North Bay stations (Napa River, Davis Point). Fig. 5 presents all total dissolved copper data for Dumbarton Bridge station, located in the South Bay, plotted over time beginning with Kuwabara et al. (1989). A temporal decline is not indicated for any of the stations within the Delta and Estuary Interface regions.

Linear regression analysis of regional data shows a significant decrease in total dissolved copper concentrations in the Southern Sloughs, as well as in the North and South Bays (Table 4). Normalized (grand mean adjusted residuals) dissolved copper concentrations have decreased 29% in the South Bay (from 32 to 23 nM). Likewise, over the past decade, total dissolved concentrations have declined from 36 to 20 nM (a 44% decrease) in the Southern Sloughs and from 29 to 24 nM in the North Bay.

3. Dissolved copper speciation

3.1. Copper toxicity and chemical speciation

Dissolved copper can exist as a variety of inorganic (Cu′) and organic (CuL_i) chemical species, and the sum of all these species is measured as the total dissolved copper concentration, $[Cu_T]$. The inorganic species of copper include the free hydrated Cu^{2+} ion, along with the complexes of copper with inorganic ligands (such as CO_3^{2-}, forming $CuCO_3^0$). Research has long demonstrated that the bioavailability of copper (as a micronutrient or as a toxicant) in freshwater and marine environments is determined by the concentration of free hydrated Cu^{2+} ion species, and not the total dissolved copper or the organically complexed copper species (Manahan and Smith, 1973; Sunda and Guillard, 1976).

The Cu^{2+} species is often described as either an *activity*, $\{Cu^{2+}\}$, or a *concentration*, $[Cu^{2+}]$. The copper ion activity, $\{Cu^{2+}\}$, is related to the copper ion concentration, $[Cu^{2+}]$, by an activity coefficient, $\gamma_{\pm2}$:

$$[Cu^{2+}] = \{Cu^{2+}\}/\gamma_{\pm2}.$$

Over the salinity range observed in San Francisco Bay, the value of this activity coefficient fluctuates between ~0.25 and ~0.3 (Morel and Hering, 1993). Using appropriate values for this activity coefficient, copper activities in the literature values have been converted to concentrations—in order to maintain consistency between early and modern copper studies conducted in San Francisco Bay.

Table 3

Temporal trends in total dissolved copper concentration at San Francisco Bay, CA Regional Monitoring Program stations, 1993–2001 (www.sfei.org)

Region	Station	p	n	$adj\text{-}r^2$	Durbin-Watson	
					d	Interpretation
Delta	Sacramento River	0.322	25	0.001	1.389	Inconclusive
Delta	San Joaquin River	0.543	24	0.000	1.596	Accept null
North Bay	Honker Bay	0.245	22	0.020	1.700	Accept null
North Bay	Grizzly Bay	0.153	25	0.047	1.461	Accept null
North Bay	Pacheco Creek	0.052	25	0.118	1.627	Accept null
North Bay	Napa River	**0.034**[a]	25	0.145	1.898	Accept null
North Bay	Davis Point	**0.035**[a]	25	0.144	1.897	Accept null
North Bay	Pinole Point	0.293	24	0.007	1.927	Accept null
North Bay	San Pablo Bay	0.383	25	0.000	2.452	Accept null
North Bay	Petaluma River	0.160	22	0.051	1.952	Accept null
Central Bay	Red Rock	0.387	22	0.000	2.218	Accept null
Central Bay	Point Isabel	0.762	25	0.000	2.716	-Serial correlation
Central Bay	Point Isabel	0.913[b]	24	0.000	—	—
Central Bay	Richardson Bay	0.178	25	0.037	2.328	Accept null
Central Bay	Golden Gate	0.952	22	0.000	1.284	Inconclusive
Central Bay	Yerba Buena Island	**0.044**[a]	24	0.135	2.185	Accept null
South Bay	Alameda	**0.008**[a]	22	0.266	1.932	Accept null
South Bay	Oyster Point	**0.018**[a]	25	0.186	1.976	Accept null
South Bay	San Bruno Shoal	**0.044**[a]	22	0.147	1.782	Accept null
South Bay	Redwood Creek	**0.009**[a]	25	0.230	1.873	Accept null
South Bay	Dumbarton Bridge	**0.002**[a]	25	0.328	2.503	Accept null
South Bay	South Bay	**0.035**[a]	25	0.144	2.324	Accept null
South Bay	Coyote Creek	**0.004**[a]	22	0.322	1.822	Accept null
Southern Sloughs	San Jose	**<0.0005**[a]	21	0.465	2.252	Accept null
Southern Sloughs	Sunnyvale	0.321	22	0.002	2.070	Accept null
Estuary Interface	Standish Dam	0.422	16	0.000	1.680	Accept null
Estuary Interface	Guadalupe River	0.542	13	0.000	1.786	Accept null

[a]Significant decrease in total dissolved copper concentrations.
[b]No trend detected after correcting for autocorrelation using the Hildreth-Lu procedure.

Table 4

Temporal trends in total dissolved copper concentrations in San Francisco Bay, CA hydrographic regions, 1993–2001

Region	p	n	$adj\text{-}r^2$	From	To	% Decline
Delta	0.233	49	0.009	—	—	—
North Bay	**<0.0005**[a]	193	0.093	28.6 nM	23.8 nM	17%
Central Bay	**0.038**[a]	118	0.028	—	—	—
South Bay	**<0.0005**[a]	166	0.246	32.2 nM	22.8 nM	29%
Southern Sloughs	**0.002**[a]	43	0.188	35.6 nM	20.1 nM	44%
Estuary Interface	0.298	29	0.004	—	—	—

Data from RMP (www.sfei.org). Normalized dissolved copper concentration estimates are presented for regions with significant temporal trends.
[a]Significant decrease in total dissolved copper concentrations.

In an inorganic system, devoid of organic copper-binding ligands, $[Cu^{2+}]$ can simply be calculated directly from the total dissolved copper concentrations $[Cu_T]$. For instance, at average pH values in seawater (pH 8.0 ± 0.3), the inorganic speciation of copper favors $CuCO_3^0$ as the dominant species, with $\sim 7\%$ of the total dissolved inorganic copper as the free hydrated Cu^{2+} ion (Turner et al., 1981; Byrne et al., 1988). At a $[Cu_T]$ of 30 nM, which is a reasonable concentration for San Francisco Bay, the

concentration of Cu^{2+} in the absence of organic chelation would be approximately 2.1 nM (or $10^{-8.7}$ M). At the EPA's national CCC guideline of 49 nM, $[Cu^{2+}]$ would be ~ 3.4 nM (or $10^{-8.5}$ M). At these $[Cu^{2+}]$, the majority of aquatic microorganisms in this environment would likely suffer from the effects of copper toxicity.

An interesting feature of studies on copper toxicity is the customary observation that organic complexation mediates toxic conditions (e.g., Sunda and Guillard, 1976; Sunda
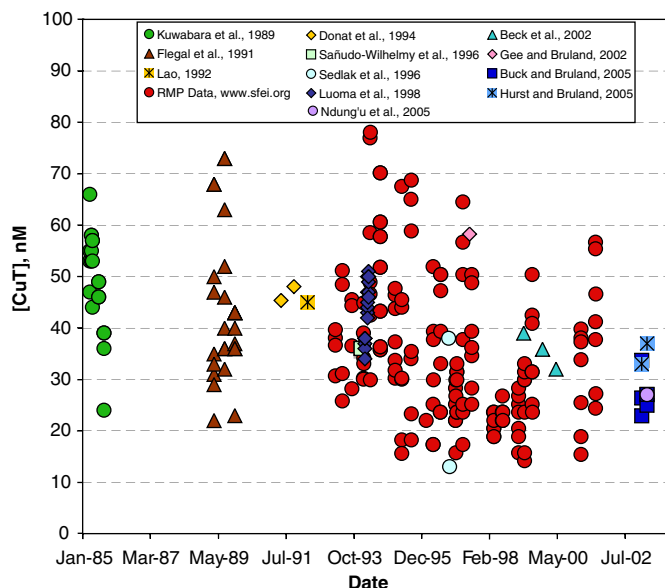
Fig. 5. Total dissolved copper concentrations measured at Dumbarton Bridge station in South Bay from 1985 to 2003.

and Lewis, 1978; Brand et al., 1986). The correlation between [DOC] and [Cu$_T$], combined with the general absence of large-scale copper toxicity events in San Francisco Bay despite elevated [Cu$_T$], suggests that organic complexation in the Bay is lowering [Cu^{2+}] below toxic levels. Thus, while strong organic ligands in the Bay lend protection against copper toxicity despite elevated [Cu$_T$], they also prevent the simple calculation of [Cu^{2+}] from the measured dissolved copper concentrations in the absence of accompanying speciation studies.

Dissolved organic matter (DOC) is a ubiquitous component of natural aquatic environments, originating from a range of sources. Copper-binding organic ligands make up a subset of this natural dissolved organic matter, tending to dominate the chemical speciation of dissolved copper in a range of marine environments (Coale and Bruland, 1988; Sedlak et al., 1997; Bruland et al., 2000; Buck and Bruland, 2005). In these environments, copper ions form thermodynamically stable complexes with "strong" (high-affinity) organic ligands, dramatically reducing the [Cu^{2+}]. In San Francisco Bay, potential sources of these ligands range from natural humic and fulvic material to urban discharge carrying anthropogenic ligands like EDTA and NTA. In addition, microorganisms which are sensitive to copper toxicity are known to be able to produce copper-binding ligands in solution themselves, further lowering the potential toxic conditions of their surrounding environment (Sunda and Huntsman, 1995; Moffett and Brand, 1996; Gordon et al., 2000).

Although recent studies have investigated the conditional stability constants and the distribution of copper-binding organic ligands in San Francisco Bay, information about their chemical structure, molecular architecture, or even important functional groups involved in copper

binding is lacking. Instead, these ligands are often described in terms of strong (L_1) and weaker (L_2) ligand classes, derived from averaged affinities of the individual copper-binding ligands present. These affinities are expressed in terms of conditional stability constants, $K^{cond}_{CuL_i,Cu^{2+}}$, which describe the equilibrium distribution of copper between the CuL$_i$ complex and the free hydrated Cu^{2+} ion, where i denotes the ligand class.

In San Francisco Bay, these two ligand classes are representative of the entire copper-binding ligand pool divided into two classes based on affinity: L_1 ligands are strong copper-binding ligands, with $K^{cond}_{CuL_i,Cu^{2+}}$ around ~10^{14} M^{-1}, and L_2 ligands are weaker, with $K^{cond}_{CuL_i,Cu^{2+}}$ closer to ~10^{12} M^{-1} (Donat et al., 1994; Beck et al., 2002; Buck and Bruland, 2005). Hurst and Bruland (2005) using a differential pulse anodic stripping voltammetric method to examine copper speciation in the Bay, argued that there is also an L_3 class of ligands present in San Francisco Bay waters that are even weaker, with $K^{cond}_{CuL_i,Cu^{2+}}$ on the order of 10^9 M^{-1}. Conditional stability constants, the theory behind them and how they are calculated in natural waters, are elaborated upon extensively elsewhere (Van den Berg and Donat, 1992; Miller and Bruland, 1997; Bruland et al., 2000; Buck and Bruland, 2005).

3.2. Copper speciation in San Francisco bay

Kuwabara et al. (1989) first demonstrated that total dissolved copper concentrations were correlated with DOC in San Francisco Bay. Approximate [Cu^{2+}] were estimated using the MINEQL computer program and external thermodynamic constants for the formation of inorganic and organic copper species (Kuwabara et al., 1989). In the absence of ambient speciation data, the program approximated that dissolved copper was likely to be between 80% and >90% organically complexed in the Bay. Wood et al. (1995) made similar estimates of extensive organic complexation with incorporation of data from Kuwabara et al. (1989) and Flegal et al. (1991) into the estuarine model ELAmet. As empirical speciation data was unavailable, estimates were based on external thermodynamic constants obtained from a laboratory study on metal interactions with humic acids (Wood et al., 1995).

In the first study to measure the chemical speciation of dissolved copper in San Francisco Bay, Donat et al. (1994) used electrochemical techniques to determine copper-binding organic ligand concentrations and their conditional stability constants, $K^{cond}_{CuL_i,Cu^{2+}}$ at a South Bay site in the spring and late summer of 1991 (Donat et al., 1994). From this work, the authors concluded that 80–92% of dissolved copper was complexed at the Dumbarton Bridge station in the South Bay. In this study, [Cu$_T$] was found to greatly exceed the [L_1], with L_1 ($K^{cond}_{CuL_i,Cu^{2+}} > 10^{13.8}$) binding only 27% of the total dissolved copper. Without a sufficiently high concentration of the strong L_1 ligands to dominate speciation, the weaker ($K^{cond}_{CuL_i,Cu^{2+}} = $~$10^{9.6}$) L_2 ligand class

appeared to play a particularly important role in the speciation of ambient dissolved copper (Donat et al., 1994).

Subsequent studies employing similar and modified electrochemical techniques have consistently determined that dissolved copper is >99.9% complexed by the strong L_1 ligand class at sites distributed throughout the North, Central, and South Bays, with the presence of a supporting L_2 ligand class also detected (Lessin, 2001; Beck et al., 2002; Buck and Bruland, 2005; Hurst and Bruland, 2005). These studies have observed that $[L_1]$ is generally in excess of $[Cu_T]$, allowing this strong ligand class to control the ambient copper speciation. In addition, a speciation study conducted in wastewater and urban discharge demonstrated that the majority of dissolved copper is likely to *enter* the South Bay already strongly complexed by organic ligands (Sedlak et al., 1997).

The incorporation of multiple analytical windows into the most recent speciation study has enabled a more thorough characterization of the large excess of L_2 that exists through the estuary (Buck and Bruland, 2005). This study found that while the strong L_1 ligands bind essentially all of the ambient dissolved copper in San Francisco Bay, a large excess of a weaker L_2 ligand exists ready to bind copper added to the system beyond the capacity of the L_1 ligand class. The dominating strong L_1 ligands and the supportive capacity of the L_2 ligand class have resulted in $[Cu^{2+}]$ below 10^{-13} M throughout the Bay (Buck and Bruland, 2005).

These high concentrations of L_1 and L_2 provide an enhanced capacity for dissolved copper, such that increases in dissolved copper concentrations must titrate these ligands before inorganic copper concentrations can increase enough to cause toxicity. This feature results in a non-linear relationship between total dissolved copper concentrations and Cu^{2+} concentrations. This relationship was examined at several sites in San Francisco Bay by Buck and Bruland (2005) and is illustrated by speciation data in $[Cu^*]$ versus $[Cu^{2+}]$ plots. $[Cu^*]$ is an operational, but useful, term that is equivalent to the amount of the total dissolved copper in solution that is bound to the ambient ligands ($[CuL_i]$). Since >99.9% of $[Cu_T]$ in these waters exists as CuL_i, $[Cu^*]$ can serve as an approximation of $[Cu_T]$, and $[Cu^*]$ versus $[Cu^{2+}]$ plots can then allow an estimate of how $[Cu^{2+}]$ will be affected by changes in $[Cu_T]$ (see Buck and Bruland, 2005 for further details).

These plots are particularly valuable in using ambient and perturbed speciation data to predict the potential impacts of changes in $[Cu_T]$ on copper toxicity (recall Cu^{2+} is the bioavailable/toxic species). Fig. 6 is an example of a Cu^* versus Cu^{2+} plot constructed from ambient copper speciation at Dumbarton Bridge in South San Francisco Bay. From this plot it is apparent that at the ambient $[Cu_T]$ (long dash line, Fig. 6) of 27 nM, $[Cu^{2+}]$ are low, $\sim 10^{-14.5}$ M. If $[Cu_T]$ were to increase to the national CCC (49 nM, short dash line, Fig. 6), we could anticipate $[Cu^{2+}]$ to increase to $\sim 10^{-13}$ M. To achieve toxic levels of Cu^{2+} ($\sim 10^{-11}$ M), this plot indicates that $[Cu_T]$ would have to exceed at least 100 nM. This capacity for increased $[Cu_T]$ before reaching
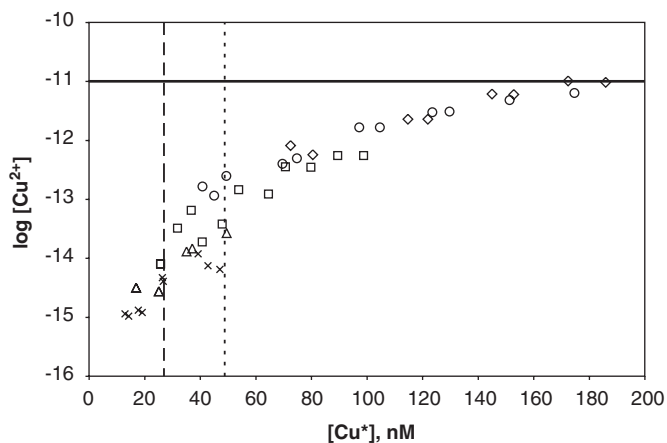

Fig. 6. The relationship between $[Cu^{2+}]$ and $[Cu^*]$: depicting the effect of increasing $[Cu^*]$ on $[Cu^{2+}]$ from titrations of Dumbarton Bridge samples at five unique analytical windows (1 μM SA: ◇; 2.5 μM SA: ○; 10 μM SA: □; 50 μM SA: △; and 100 μM SA: X). The short and long dashed lines correspond to the national CCC of 49 nM and the ambient $[Cu_T]$ of 27 nM, respectively. The horizontal shaded line presents the possible toxicity threshold for ambient microorganisms of 10^{-11} M Cu^{2+} (from Brand et al., 1986).

toxic $[Cu^{2+}]$ is attributed to the ambient organic ligands present (Buck and Bruland, 2005). Investigations into a more appropriate *site-specific* objective (SSO) for the Bay have further demonstrated this capacity in ambient South Bay waters near Dumbarton Bridge, where approximately 108 nM (6.9 μg/L) of total dissolved copper could be reached before toxicity to aquatic microorganisms was observed (Tetra Tech Inc. et al., 2000).

The dominance of the CuL_1 species in San Francisco Bay has dramatically reduced concentrations of the biologically available Cu^{2+} ion. Beck et al. (2002) evaluated the bioavailability of this species in the Bay with laboratory incubation experiments conducted in ambient South Bay water collected during the annual spring diatom bloom (Beck et al., 2002). The CuL_1 species was found to be relatively inert to the ambient phytoplankton and was not significantly assimilated (Beck et al., 2002), supporting the earlier observations of Luoma et al. (1998) that dissolved copper concentrations were minimally affected by the 1994 spring diatom bloom. The inert nature of these CuL_1 complexes, even during a marked phytoplankton bloom, facilitates the persistence of elevated dissolved copper concentrations in the Bay (Beck et al., 2002).

This dominance by L_1 observed in the Bay since 2000 (Lessin, 2001; Beck et al., 2002; Buck and Bruland, 2005; Hurst and Bruland, 2005) is in contrast to the 1991 speciation results of Donat et al. (1994), where L_1 concentrations were lower than total dissolved copper concentrations, and L_2 (or even L_3) ligands were weakly binding the majority of dissolved copper in San Francisco Bay. The analytical methods employed in these studies are comparable to the more recent work, and there is no indication that the discrepancy in the data originates from the methodology. Of note, while a very strong added ligand

(8-hydroxyquinoline) was used in the 1991 work, a sufficiently low concentration was incorporated to ensure comparable analytical windows (Donat et al., 1994). In using a strong added ligand, the resulting analytical window can only accurately characterize the strong ambient ligand class (Donat et al., 1994), and multiple analytical windows must be incorporated in order to distinguish the weaker ligand classes (Buck and Bruland, 2005).

In the 1991 studies the free hydrated $[Cu^{2+}]$ was up to $10^{-9.7}$ M, and this level of $[Cu^{2+}]$ would be expected to cause toxicity to the ambient cyanobacteria (Donat et al., 1994). At the time of this study, no information on the cyanobacterial populations in the Bay was available, although Cloern (1996) noted in a review of phytoplankton dynamics in the Bay that cyanobacteria were "not commonly found" here. More recent data from the summer of 1999 has demonstrated that cyanobacteria flourish in the South Bay (up to 50,000 cells/mL), indicating these waters are not toxic (Palenik and Flegal, 1999). While it is tempting to draw conclusions from this data, we suggest that there is insufficient evidence to prove that there has been a marked change in concentrations of $[L_1]$ and dissolved copper speciation since that first speciation study in San Francisco Bay.

3.3. Copper-binding ligand sources

The ambient ligands that bind copper in the marine environment have not yet been structurally characterized, making it challenging to discuss the sources of these ligands to San Francisco Bay. Instead, we will structure this discussion around reported conditional stability constants $(K^{cond}_{CuL_i,Cu^{2+}})$ for the ambient ligands and the known copper-binding components that may serve as these ambient ligands. Among the potential sources of copper-binding ligands to the Bay, notable possibilities include wastewater organic matter (WWOM), biopolymers from activated sludge and raw sewage components, natural humic and fulvic acid input and exudates from ambient phytoplankton.

The L_1 ligands observed in San Francisco Bay have a strong affinity for copper, with a $K^{cond}_{CuL_i,Cu^{2+}}$ of $\sim 10^{14}$ M^{-1}. Exudates from phytoplankton, specifically from the cyanobacteria that are most sensitive to copper toxicity, may constitute a portion of the strong L_1 ligand class for copper. Laboratory studies have observed that the ligands exuded by Synechococcus species under copper stress have high affinities for copper ($K^{cond}_{CuL_{Syn},Cu^{2+}} > 10^{12}$ M^{-1} Gordon et al., 2000; $K^{cond}_{CuL_{Syn},Cu^{2+}} = 10^{12.3-13.3}$ Moffett and Brand, 1996), in the range of ambient L_1 ligands. Previous work has shown that WWOM ($K^{cond}_{CuWWOM,Cu^{2+}}$ up to $10^{13.8}$ M^{-1} Sarathy and Allen, 2005; $K^{cond}_{CuWWOM_1,Cu^{2+}}$ up to $10^{14.5}$ M^{-1} Sedlak et al., 1997), including synthetic chelators like EDTA ($K^{cond}_{CuEDTA,Cu^{2+}} = 10^{13}$ M^{-1} Sedlak et al., 1997), have high affinities for copper in freshwater, although the

binding strengths of this organic matter is expected to decline in the more saline waters of the Bay with the increased competition from Ca^{2+} and Mg^{2+} in the seawater (e.g., $K^{cond}_{CuEDTA,Cu^{2+}}$ for EDTA in seawater $\sim 10^{10}$ M^{-1}). Thus, ligands from these sources are more likely to contribute to the weaker L_2-type binding observed in the Bay.

The weaker L_2 ($K^{cond}_{CuL_i,Cu^{2+}} \sim 10^{12}$ M^{-1}) and L_3 ($K^{cond}_{CuL_i,Cu^{2+}} \sim 10^9$ M^{-1}) ambient ligands in the Bay generally exist at higher concentrations than their stronger counterparts and may come from some of the same sources as the L_1 ligands, as well as from distinct sources of their own. In their work, Sedlak et al. (1997) have observed a weaker ligand class within the WWOM ($K^{cond}_{CuWWOM_2,Cu^{2+}} = 10^{6.8-7.3}$ M^{-1}), which may be attributed to biopolymers from activated sludge or even humics. Raw sewage has been shown to bind copper weakly at pH 7.8 ($K^{cond}_{CuRS,Cu^{2+}} = 10^{8.7-10.6}$ M^{-1}, Kunz and Jardim, 2000) and natural exudates from eukaryotic phytoplankton and heterotrophic bacteria present a similarly weak affinity for dissolved copper ($K^{cond}_{CuL_{Ditylum},Cu^{2+}} \sim 10^{9-12}$ M^{-1}, Rijstenbil and Gerringa, 2002; $K^{cond}_{CuL_{Vibrio},Cu^{2+}} \sim 10^{7.9-11}$ M^{-1}, Gordon et al., 2000). Humic and fulvic acids exhibit a wide range of binding affinities for copper, from $\sim 10^7$ M^{-1} (Lorenzo et al., 2006) to $\sim 10^{12}$ M^{-1} (Kogut and Voelker, 2001) and are likely to constitute some fraction of the weaker ligands observed in these waters—particularly in North Bay where freshwater input is high and where the surrounding marshlands may be an internal source of these compounds.

In the absence of chlorophyll and DOC data collected during speciation exercises, it is difficult even to constrain the possible sources. In future speciation studies, the inclusion of chlorophyll-a and DOC measurements may enhance our understanding of copper-binding ligand sources and cycling, and the role of these ligands in governing toxicity.

4. Conclusions and future implications

Total dissolved copper concentrations in the North and South San Francisco Bay have significantly declined since the implementation of the San Francisco Estuary Institute's Regional Monitoring Program for Trace Substances in 1993. Concentrations in the Southern Sloughs have declined the most, with a 44% decrease in total dissolved copper concentrations. Dissolved copper concentrations are positively and significantly correlated with DOC at stations throughout the Bay, and 53% of the variability in concentrations is accounted for by a combined model of DOC and daily Delta outflow.

The relationship between dissolved copper concentrations and DOC is supported by speciation analyses conducted in the Bay. These speciation studies have demonstrated that $>99.9\%$ of the dissolved copper in

San Francisco Bay is currently complexed by strong copper-binding organic ligands in the form of CuL_1, with a large pool of weaker ligands present to potentially buffer the system against small seasonal changes in dissolved copper concentrations. Additionally, the CuL_1 complex appears to be relatively inert with respect to particle scavenging, adsorption, and biological uptake.

Exchange between the dissolved and particulate phases is very important, and this exchange acts as a primary source of dissolved copper in South Bay (Gee and Bruland, 2002). However, while the distribution of copper between exchangeable particulate phases and dissolved phases is provided by the RMP, interpretation of processes inherent in this cycling requires additional kinetic data (Gee and Bruland, 2002). These kinetic measurements must take into account the features of this system (Gee and Bruland, 2002), including, at a minimum, the strong organic complexation both within San Francisco Bay (Beck et al., 2002; Buck and Bruland, 2005), but also within point source inputs (Sedlak et al., 1997).

While the origins and molecular structures of the copper-binding ligands remain largely unknown, the strong organic complexation of dissolved copper in the Bay has reduced concentrations of the free hydrated copper ion, the toxic species of copper, to below 10^{-13} M. At these low levels it is highly unlikely that copper poses any toxicity threat to ambient aquatic microorganisms in San Francisco Bay. Without these copper-binding organic ligands, however, Cu^{2+} would exist at levels toxic to most microorganisms living within the Bay.

Acknowledgments

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