Linkage of Bioaccumulation and Biological Effects to Changes in Pollutant Loads in South San Francisco Bay

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The developed world has invested billions of dollars in waste treatment since the 1970s; however, changes in ecological or biological responses are rarely associated with reductions in metal pollutants. Here we present a novel, 23-yr time series of environmental change from a San Francisco Bay mudflat located 1 km from the discharge of a suburban domestic sewage treatment plant. Samples of surface sediment, the bioindicator Macoma balthica, and metals loading data were used to establish links between discharge, bioaccumulation, and effects. Mean annual Ag concentrations in M. balthica were 106 μg/g in 1978 and 3.57 μg/g in 1998. Concentrations of Cu declined from 287 μg/g in 1980 to a minimum of 24 μg/g in 1991. Declining Cu bioaccumulation was strongly correlated with decreasing Cu loads from the plant between 1977 and 1998. Relationships with bioaccumulation and total annual precipitation suggested that inputs from nonpoint sources were most important in controlling Zn bioavailability during the same period. Ecoscience and biological responses are rarely associated with changes in metal bioavailability. In some aspects of regional contamination in San Francisco Bay were more severe in the 1950s to 1970s than in the 1990s (4). However, environmental concentrations of metals alone do not allow evaluation of changes in biological effects. Thus, uncertainty remains about the ecological benefits of regulation and reduction of heavy metal inputs. The uncertainty partly stems from the temporal resolution of the existing data. Few data sets incorporate the time span during the period immediately after the implementation of the Clean Water Act. Monitoring in receiving waters near local discharges is rare in most coastal waters, and in cases where it does exist, the programs are less than 1 decade old (5, 6). Uncertainty also stems from the inherent difficulty of proving adverse effects of metal pollutants in complicated natural settings (7). Historic biological or ecological data are rarely adequate to evaluate change, especially where changes in metal exposures are greatest. Few case studies exist that link changing biological processes to changing metal exposures. Sindermann (8) listed several ecopaleontological criteria necessary to evaluate the probability that an associative link between an environmental stressor and a biological response reflects cause and effect. The criteria include strength and consistency of the association, specificity of the association, temporal relationship, and plausibility and probability of the hypothesis. Carefully conducted time series from nature, across a period of effect and recovery, can be an effective means to meet some of these criteria. Adequate characterization of trends in exposure is essential in evaluating if changes in biology or ecology are linked to contamination. Sediment and benthic organisms are useful in determining spatial distributions and temporal trends of exposure (9, 10). Many metals bind strongly to fine-grained (silt/clay) sediment, and a record of metal release to an environment can be retained (11). Although controversial in some quarters (12), analysis of the tissues of organisms (bioaccumulation) complements sediment analyses and can be an effective means of estimating trends in bioavailable metal exposures (9, 10). Ideally, one resident species must be analyzed consistently over time to interpret bioaccumulation trends, but extrapolation of exposures to other food web organisms is usually reasonable (11).

Presented here is a novel, 23-yr data set from a mudflat located 1 km from the point of discharge of a suburban domestic sewage treatment plant. The data include characterization of local environmental conditions such as sediment chemistry, food availability, hydrologic conditions, metals bioaccumulation in a resident bivalve (Macoma balthica), and point source loadings. Reproductive activity in M. balthica was measured during four time segments early in the study, providing a measure of biological response that occurred through time. The intensity of sampling allowed characterization of seasonal variability in metal exposure and, most important, long-term biological responses to environmental changes. The magnitude of the decrease in point source loadings since 1977 and the corresponding improvements of reproductive success in M. balthica link historic elevated metal exposures with adverse effects on the resident bivalves.

Methods

The study site is located in south SFB, a large coastal embayment with a watershed dominated by urban development (Figure 1). The major freshwater inputs are small local stream inflows and wastewater discharges during most of the year (13). Urban runoff is discharged into the local streams and then carried to the Bay untreated during the rainy season.
FIGURE 1. Site location map.

(typically characterized by the winter months, December—April). South SFB has large shallow expanses less than 2 m deep (13). Strong diurnal winds, which are at a maximum from June through August, mix, resuspend and transport sediment out of the shallows through the summer (14). Runoff from local streams and the larger rivers replenish this sediment annually during winter. Because of the yearly renewal and resuspension of sediment, continual deposition is not characteristic of the intertidal zone in South Bay (15).

The resident bivalve, *M. balthica*, and surface sediment were collected near-monthly and within 20 m of each other from a mudflat 1 km south of a municipal discharge source in south SFB (Figure 1). The period of record for *M. balthica* is from 1975 to 1998. Surface sediment was first collected in 1977. Frequent sample collection and the long period of record were instrumental in discerning differences between seasonal variation and interannual change. Field and analytical procedures for surface sediment and *M. balthica* have been presented in detail elsewhere (16, 17). The oxidized layer of sediment (approximately 1–2 cm) was collected from the surface of the mudflat. Sediment samples were wet-sieved through a trace metal clean 100-μm polyethylene mesh with ultrapure deionized water. This size best represents the largest size particles that are ingested by *M. balthica* (16). Sieving has the further advantage of eliminating the bias that might occur as a result of coarser particles that accumulate during the summer months (18).

Sediment metal concentrations were determined using a near-total digest by concentrated HNO₃ reflux (for Cu and Zn) and a 2-h extraction with weak acid (0.6 N HCl) for Ag using established procedures (4). The near-total digest does not provide complete recovery of all metals, but it is indicative of metals sufficiently mobile to be of toxicological interest (19) and is comparable to other data from the Bay and elsewhere (4, 17). The weak acid HCl extraction was used to determine the bioavailability of Ag from sediment (20).

The deposit feeding clam, *Macoma balthica*, was collected simultaneously with the sediment. At least 40 individuals were collected on most occasions. After depuration for 48 h (16), clams were sorted into 1-mm size ranges. Each monthly sample consisted of approximately 6–13 composites; each composite consisting of 3–7 animals of similar shell length. Animal tissues were dried, weighed, and refluxed in concentrated HNO₃ until the digest was clear. Digests were evaporated to dryness, reconstituted in 0.6 N HCl, and filtered through a 0.45-μm filter.

Standard Reference Materials were used to determine percent recoveries and were analyzed at regular intervals throughout the study (18). Reference material used over the period of record included oyster tissue, albacore tuna, bovine liver, and estuarine sediment. Between 1975 and 1989, Ag, Cu, and Zn in tissues and sediment were determined by flame atomic absorption spectroscopy (AAS). Since 1990, with the exception of Ag in sediment, metals in both sediment and *M. balthica* were determined by inductively coupled argon plasma emission spectroscopy (ICPES) after correction for matrix-specific interference. Silver concentrations in sediment were determined by graphite furnace atomic absorption spectrophotometry (GFAAS) with Zeeman background correction and standard additions technique. The change in analytical methodology was accompanied by extensive cross-checking to ensure that all data were comparable. Careful application of QA/QC techniques, including the evaluation of both reference materials and percent recoveries over time, confirms that the methods employed were consistent (18).

Because loadings from storm runoff into South Bay are not well-known, precipitation was used as a surrogate for inputs from urban runoff. Changes in salinity can also affect metal speciation and bioavailability (21–23) but did not appear to be an important cause of changes in Ag and Cu bioaccumulation (24) and, therefore, are not reported here. Precipitation data were collected by the National Weather Service at a station within the city limits of Palo Alto. Metal loads associated with discharge from the Palo Alto Regional Water Quality Control Plant (PARWQCP) were analyzed by plant personnel. Metal concentrations in effluent were determined by flame AAS until 1989. Because of the high concentrations of Cu and Zn in the plant effluent, data collected prior to 1989 are probably reliable, but Ag data are questionable. However, after 1989, analyses were conducted by GFAAS, and presumably all data are reliable. To provide a comparable scale to the bioaccumulation data, all pre-
cipitation and loadings data are presented by calendar year.

Total organic carbon (TOC) was measured using a carbon analyzer on sieved sediment by methods outlined by Thomson-Becker and Luoma (25). Chlorophyll a fluorescence was measured independently of this study, using a Sea Tech submersible pulsed-light fluorometer (26).

Reproductive stage was determined using stained thin sections of the visceral mass of preserved specimens of M. balthica that had been collected at a site ~500 m north of the Palo Alto mudflat between 1974 and 1998 in a separate study (27). The method used is described in Parchaso et al. (28). Four periods of data were available from near-monthly collections: February 1974–July 1975; June 1979–October 1981; January 1983–February 1985; and January 1988–September 1989 (27). Only animals of reproductive age were collected for sectioning, and on most dates, 10 clams were examined. Gonadal development was characterized in two categories: gonadal cells containing mature gametes (28) and gonadal cells in which gametes were either not apparent or had follicles that had not achieved reproductive maturity.

Results
Trends in PARWQCP Inputs. The PARWQCP serves a suburban community with some light industry (electronics, small plating facilities; a photographic facility that was operational until the mid 1980s). Its history is similar to that of other municipal dischargers in the United States. Secondary treatment was installed after passage of the Clean Water Act in 1972. In 1980, advanced treatment was added, including trickling filters (which allowed nitrification to occur in the preexisting aeration tanks) and dual media filters (17). In 1988–1989, clarifiers were added, improving solids removal, especially during wet weather. Source control programs for other municipal dischargers in this region had been in effect since the late 1970s, averaging 98.2 ± 10.4 liters/day (Figure 2). Copper concentrations in the effluent as high as 150 μg/L were determined in the late 1970s, and estimated total loadings reached a peak of approximately 5800 kg/yr in 1979 (Figure 2). Concentrations of Cu in 1998 averaged 7 μg/L, and total annual loadings were 263 kg/yr (Figure 2). Concentrations of Ag in the effluent have also declined. In 1989, average Ag concentrations were 3.0 μg/L and have since decreased to 0.2 μg/L. Silver loadings decreased from 91.6 kg/yr (1989) to 7.7 kg/yr in 1998 (Figure 2). Zinc concentrations and loads have not changed in the effluent, although concentrations were more stable after the upgrades. In 1998, mean concentrations in the effluent were 49 mg/L, and loadings were 1903 kg/yr. Presumably the lack of change reflects the low degree of Zn contamination historically associated with this suburban effluent.

Environmental Characteristics. Factors that could potentially influence metal fate and effects were also measured during the course of the study. These include precipitation, sediment geochemistry, and indicators of food availability. Seasonal wet (December–April) and dry (May–November) periods (Figure 3) characterize the pattern of rainfall. Although a combination of wet and dry years occurred during the 23-yr time frame, there was no long-term unidirectional trend in precipitation. Pronounced periods of low precipitation occurred in 1976–1977, 1984–1985, and 1987–1991 (Figure 3). Strong ENSO (El Niño) events in 1982–1983 produced one of the wettest years of this century. High precipitation and inflows also occurred in 1986, 1993, 1995, 1996, and 1998. Monthly salinity varied from 5 to 33 at the Palo Alto mudflat over the 23-yr study period and generally followed rainfall patterns (17).

The geochemical nature of the sediment, indicated by total organic carbon, Fe, and Mn, showed no significant unidirectional, long-term trends on the mudflat (Figure 3). The percent TOC in the Palo Alto mudflat averaged 1.24 ± 0.31% between 1977 and 1998, with a range of 0.4–2.1% dry weight. No unidirectional change was evident as the PARWQCP cleaned its effluent, presumably because a large discharge of organic material was not a problem at this suburban plant after the mid-1970s. Concentrations of near-total Fe averaged 4.4 ± 1.2% between 1977 and 1998, and Mn concentrations averaged 1650 ± 370 μg/g (Figure 3). Although some year-to-year fluctuation of redox-sensitive Fe and Mn occurred in the oxidized surface sediment, most specifically in response to the wettest years, concentrations did not progressively change during the study period. Sediment was visibly oxic at the surface over the entire period of record.

The availability of food to M. balthica probably did not change greatly over the 2 decades. The magnitude and frequency of phytoplankton blooms varied from year to year but showed no progressive trends (Figure 3) during the course of the study.

Trace Metal Trends. Silver concentrations in surface sediment decreased by 3-fold between 1977 and 1998 (Figure 4). Although concentrations of Ag were at their highest in 1979 (annual mean of 3.6 ± 0.42 μg/g), they did not exceed the effects range-median (ERM) value of 3.7 μg/g (29). Silver declined to the lowest levels in the mid-1990s (e.g., 0.20 ± 0.14 μg in 1991). Concentrations of Cu in surface sediment also decreased by half over the study period, from an annual mean of 86 ± 28 μg/g in 1979 to 43 ± 10 μg/g in 1993 (Figure 4). Annual mean Cu concentrations in the sediment remained significantly lower than the ERM value throughout the course of the study (Figure 4). In contrast, annual mean Zn concentrations in bed sediment showed no distinct trends over time, with a 21-yr annual average of 145 ± 37 μg/g (Figure 4). Zinc concentrations in the sediment have remained consistently close to the effects range-low (ERL) value of 150 μg/g (29) during this time period. In the mid-1980s, annual mean Ag concentrations declined to nearly half the ERL value of 1.0 μg/g. Copper concentrations were slightly above the ERL value at this time (Figure 4). Although significant decreases in Ag and Cu concentrations in the sediment did occur over time, concentrations for both metals were still 2–3-fold higher than regional background concentrations (4) in 1998.

Concentrations of Cd, Cr, Hg, Ni, Pb, Se, and V have been analyzed in sediment since 1990 and were also determined.
in selected archived samples from the 1970s and 1980s. None of these metals were elevated in concentration to the extent of Ag and Cu, and significant temporal trends in concentrations of these metals were not found (17). The limited historic contamination by these elements may reflect the largely suburban nature of the watershed.

Mean annual concentrations of both Ag and Cu in *M. balthica* showed a strong trend of declining concentrations from the 1970s through 1991 (Figure 4), despite high, distinctly seasonal, intra-annual variability (30). The magnitude of seasonal variation, represented by the error bars, is a product of seasonal tissue growth and tissue metal concentrations. This is proportionately consistent among years (17). The highest annual mean concentration of Ag in *M. balthica* during the study was 109 ± 41 µg/g in 1980; Ag concentrations were 3.7 ± 0.8 µg/g in 1996. The highest and lowest Cu concentrations in *M. balthica* averaged 340 ± 119 µg/g in 1975 and 24 ± 13 µg/g in 1991, respectively. The annual mean concentrations of Ag in *M. balthica* appeared to respond rapidly to the treatment upgrades that occurred at the PARWQCP. For example, mean concentrations of Ag declined more than 50% between 1980 (109 ± 41 µg/g) and 1982 (45 ± 22 µg/g) after the first phase of plant improvements. They again declined >50% between 1987 (55 ± 30 µg/g) and 1989 (11 ± 7 µg/g), after the second phase. Copper concentrations show a more gradual decline over time (Figure 4).

Like sediment, no unidirectional trend was observed in annual mean Zn concentrations in *M. balthica* between 1977 and 1998 (Figure 4). Annual average concentrations of Zn in *M. balthica* were 277 ± 31 µg/g in the mid- to late-1970s. Concentrations increased in the early 1980s to 382 ± 74 µg/g, declined in 1987–1991 (212 ± 57), and then increased after 1991 to 434 ± 94 µg/g in 1996.

Although concentrations of Cu and Ag in *M. balthica* declined substantially after the 1980s, concentrations in the 1990s were not as low as the regional background levels in this species. The mean regional baseline for Cu in *M. balthica* is about 25 µg/g (17, 31) as compared to a mean Cu concentration of 47.2 ± 16.7 µg/g observed at Palo Alto from 1990 to 1998. Regional baseline concentrations of Ag and Zn were 0.5 and 200 µg/g, respectively (17, 31).

**Influence of Environmental Variables.** Bioaccumulated concentrations of Ag and Cu were significantly related to sediment metal concentrations among all data (Table 1). This relationship appeared to be driven primarily by the high concentrations observed in the sediment between 1977 and 1988. There was no significant relationship in the 1989–1998 data alone, when sediment metal concentrations were low. Concentrations of Zn in sediment were not significantly correlated with concentrations in *M. balthica*, probably due to the small range in concentrations during the study period (Figure 4).

Concentrations of Cu and Ag in *M. balthica* between 1977 and 1998 were also linked to metal loads from the PARWQCP (Table 1). Calculated annual Cu loads from the plant were strongly correlated with annual mean Cu concentrations in *M. balthica* for the entire study period (Table 1; Figure 5). However, between 1989 and 1998 alone, this relationship was not evident because while Cu loads declined steadily from 587 to 263 kg/yr (Figure 2), Cu concentrations in *M. balthica* were near background at 24 ± 13 µg/g in 1991, increased to 73 ± 17 µg/g in 1996, and decreased again during the next 2 yrs (Figure 4).

Silver concentrations in *M. balthica* also corresponded to loads (Table 1; Figure 5), but this relationship can only be tested from 1989 to 1998 because of the absence of reliable Ag loading data prior to 1989. It is reasonable to assume that the relationship was strong prior to 1989 since the PARWQCP was the primary source of Ag loadings to the mudflat (16).
Nonpoint source influence on Zn contamination was indicated by the correlations. Fluctuations in Zn in M. balthica did not correspond to fluctuations in Zn loadings from the PARWQCP. But, a significant, positive relationship between precipitation, a proxy for inputs from urban runoff, and Zn bioaccumulation was observed for the entire time series (Table 1; Figure 5). It is unlikely that speciation changes associated with freshwater inflows caused the precipitation-
related trends in Zn. Salinities rarely reached the low values necessary to greatly affect speciation of any of these elements (32). The relationship of Ag or Cu bioaccumulation with precipitation was not significant when compared to annual mean concentrations in M. balthica between 1977 and 1998 or between 1989 and 1998 (Table 1).

**Discussion**

The study site near the PARWQCP was characterized by only moderately contaminated sediment, as defined by Long et al. (29), but severe Ag and Cu contamination in the tissues of *M. balthica* occurred in the late-1970s and early-1980s (16). Contamination with Ag and Cu was also documented in resident biota elsewhere in south SFB in the 1980s (24, 30) but to a lesser extent than at Palo Alto. Inputs from other dischargers in South Bay do not greatly influence the Palo Alto site (18, 31), although all inputs together probably generate regional-scale contamination. Stemberson and Leonard (33) also reported declining Ag concentrations (but not Cu) in transplanted mussels (*Mytilus californianus*) at a California mussel watch during the period between 1977 and 1990.

Sediment cores collected in SFB show that concentrations of Ag and Cu peaked between 1965 and 1975 but have decreased since the 1980s (4). The biological data presented here also suggest that these metals may be decreasing in the Bay. However, it is interesting to note that dissolved Ag and Cu concentrations remain higher in south SFB than found elsewhere in the Bay (2, 34). For example, in 1990, dissolved Ag concentrations were equivalent to those found in the highly contaminated San Diego Bay (35). This combined with the historical signature of metal evident in the sediment suggest that it is reasonable to expect that Ag and Cu concentrations in *M. balthica* will continue to exceed regional background concentrations.

High tissue body burdens of Ag and Cu in *M. balthica* show exposure to bioavailable contamination at an extreme level prior to 1983 with declining concentrations thereafter. The reproductive trends link the high Ag and Cu exposure with an absence of mature gametes in the resident animals and show a subsequent recovery from that adverse effect as metal exposures declined. By itself, the coincidence of these two factors does not prove a cause and effect relationship however. Sindermann (9) defined ecologic criteria...
that aid characterization of the probability of causality; these criteria can be applied to the present study.

**Temporal and Statistical Association.** Although the temporal relationship described by Sindermann (8) is exposure, preceding response, our data illustrate an alternative opportunity to observe a temporal association: declining exposure followed by recovery. The low reproductive potential of *M. balthica* coincided with the period of highest Ag and Cu bioaccumulation. As metal bioaccumulation became less severe, the proportion of mature gametes in *M. balthica* increased. Both data sets are extensive among years and intensive within years; thus, the coincidence was not a result of sampling or analytical error.

Annual average concentrations in Ag and Cu were significantly correlated with the annual average percent of *M. balthica* with mature gametes (Figure 7; $R^2 = 0.35$ and 0.45, $p < 0.05$, respectively). When Ag concentrations in *M. balthica* in this data set were greater than 70 µg/g, the percent of mature gametes found in *M. balthica* did not exceed 35%. As Ag loads were reduced and Ag bioaccumulation reached more moderate levels (20–60 µg/g), the percent of animals with mature gametes began to increase (Figure 7). Elevated Cu concentrations also suggest a relationship with reproduction in *M. balthica*. When Cu bioaccumulation was high, *M. balthica* did not contain high percentages of mature gametes. Only when Cu bioaccumulation in this data set decreased to levels ≤100–200 µg/g did the reproductive pattern improve (Figure 7).

**Specificity of the Association.** Many factors can affect or interfere with reproductive capabilities in bivalves. However, our analysis decreases the likelihood that the observed effect was associated with other environmental parameters. Reproductive impairment did not co-vary with food availability (there was no relationship with TOC or phytoplankton blooms). The sediment chemistry and dissolved oxygen availability at the study site remained relatively stable over time, as evidenced by the Fe and Mn concentrations and the consistent observations of oxic sediment. While seasonal fluctuations in salinity or precipitation occurred within a year, long-term trends did not co-vary with the observed trends in reproduction. Other metals often associated with point-source discharge did not show significant long-term temporal trends (17).
A number of organic contaminants were not measured as part of this study. Most, but not all, can be discounted as a possible cause of the observed effect on the basis of trends. PAHs have not declined in SFB (36), and modern pesticide usage has increased since 1980 (37). Although TBT has been banned, TBT-specific transplant bioassays in the 1980s indicate that TBT was not a problem near Palo Alto (38). Regionally, elevated concentrations of organochlorines followed regional trends somewhat similar to Ag and Cu and are persistent in SFB sediments (37). However, because M. balthica was abundant elsewhere in the Bay (24, 27), it can be inferred that reproduction was not inhibited by regional organochlorine contamination during this time period. We cannot eliminate the possibility that our site was an organochlorine hotspot in the 1970–1980s, but there is no land use evidence to support existence or remediation of such a hotspot. One can never eliminate every possible confounding variable in a field study, but the most likely alternative causes of such change, other than exposure to Ag and/or Cu, do not appear to show the necessary associations.

Strength and Consistency of the Association. Coincident with the high Ag and Cu exposures, the thin tissue sections clearly showed that M. balthica were rarely able to produce mature gametes between 1974 and 1981. An abnormal reproductive cycle (28) occurred at least periodically through 1988. When the clams were not successfully producing gametes, they most likely were not reproducing successfully at Palo Alto. M. balthica has a pelagic larval stage that could be carried to Palo Alto from reproducing populations at less contaminated sites elsewhere (24). Some selection of individuals tolerant to Cu and Ag may also have occurred from the assemblage of immigrating recruits, aiding local survival. Thus, it is possible that immigration and selection for tolerance allowed persistence of M. balthica in this contaminated habitat despite the low likelihood that they were reproducing there.

Coherence and Plausibility of the Association. Laboratory tests have demonstrated that reduced reproductive capabilities in other marine invertebrates can be linked to elevated levels of Cu (39, 40) and Ag (41). Thus, this specific effect is plausible in invertebrates, although the biochemical mechanisms have not been studied. In addition, this effect occurs at an exposure level in sediments recognized for at least low-level toxic effects (at values above the ERL but below the ERM). Plausibility is also enhanced when other physiological signs of metals-related stress are evident. In the present study, several lines of evidence associate highly elevated exposures to Cu and Ag with physiological and biochemical stress responses in M. balthica. In 1980, Johannsson et al. (42) observed metal saturation of the metallothionein (MT) pool in M. balthica at the Palo Alto study site. High tissue concentration, and a “spillover” of Cu and Ag into the lower molecular weight protein pool, was observed. This has been shown as a sign of metal-specific biochemical stress in other organisms (43). Also in 1980, it was observed that the population of M. balthica at Palo Alto was tolerant to Cu (and Ag, unpublished), presumably related to synthesis of MT as compared to populations from less contaminated mudflats (44). This is also diagnostic of metal-specific stress (45).

Although it is not possible to establish unequivocal proof of a cause and effect relationship based solely on field measurements, we have identified a probable association between Ag and Cu bioaccumulation and disruption of reproduction in a marine bivalve. The probability of causation is relatively high based upon the satisfaction of a set of ecotoxicological criteria for establishing cause and effect. That these results were evident when Ag and Cu concentrations in the sediment were in the range between the ERL and the ERM values suggest that chronic exposure may lead to adverse population level effects in at least some moderately contaminated environments. Confirmation of the conclusion that metals can chemically disrupt reproduction requires further mechanistic studies of this effect.

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