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Influence of seasonal growth, age, and environmental exposure on Cu and Ag in a bivalve indicator, *Macoma balthica*, in San Francisco Bay

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ABSTRACT: Temporal and spatial variations in Cu and Ag in the deposit-feeding clam *Macoma balthica* and in surficial sediments were analysed at 8 stations in San Francisco Bay at near-monthly intervals for periods ranging from 3 to 10 yr during 1977 to 1986. Strong seasonal variations in metal concentrations of *M. balthica* were associated with seasonal variations in soft tissue weight. Aperiodic fluctuations in metal concentration appeared to be driven by changes in metal content of the soft tissues. Metal content of clams of standard shell length was less variable than tissue metal concentration, and generally followed changes in the concentrations of Cu and Ag in the sediments. Correlations between metal content and sediment concentrations were improved when content was standardized to age rather than shell length. Metal content of *M. balthica* displayed few consistent temporal trends among stations, evidently reflecting different sources of input and complex hydrologic and geochemical processes affecting metal availability in San Francisco Bay. Increases in Cu and Ag were noted at several stations in South Bay during 1977 to 1980. A continuous 10 yr record at one of these stations showed that the 1977 to 1980 increase and the subsequent decline beginning in 1981 coincided with fluctuations in metal inputs from a nearby source.

INTRODUCTION

Bivalve molluscs are routinely employed to define the temporal and spatial distributions of biologically available environmental pollutants, including trace metals, in coastal areas and estuaries (Goldberg et al. 1978, Farrington 1983, Bryan et al. 1985). Interpretation of when and where contaminant concentrations in the bodies of organisms reflect environmental exposure, however, can be complicated by biological processes such as seasonal growth, reproductive cycles, and age (Phillips 1976a, Riisgård et al. 1985, Borchardt et al. 1988). Experimental studies have demonstrated the dependence of metal bioaccumulation on exposure concentrations (Zamuda & Sunda 1982, Amiard et al. 1987, Mason et al. 1988), and the importance of considering biological processes in data interpretation (Simpson 1979, Fischer 1988). However, the application of such knowledge will require better descriptions of the relative influences of biological and environmental factors in field studies of bioaccumulation.

Various procedures have been suggested to overcome the biases in tissue metal concentration data introduced by biological processes (Phillips 1976a, Simpson 1979, Clifton et al. 1983, Fischer 1983, Cossa & Rondeau 1985). To directly analyse the pattern of temporal variability in bioaccumulated Cu that could result solely from seasonal fluctuations in tissue mass that occur during an annual growth cycle in the bivalve *Macoma balthica*, Cain & Luoma (1986) employed an empirical model that varied tissue metal concentrations inversely with changes in dry tissue weight. This hypothetical pattern of variation closely matched the observed variation in metal concentrations at 3 of 4 stations examined from which 3 to 5 yr of near-monthly data were available.

In San Francisco Bay, California, USA, temporal fluctuations in the concentrations of trace metals in the soft tissues of the clam *Macoma balthica* appear to be driven by a combination (1) localized, episodic metal inputs, (2) hydrologic processes that affect both the distribution of metals in the Bay and their bioavailability, and (3) seasonal growth of the clam (Luoma & Cain 1979, Strong & Luoma 1981, Luoma et al. 1985). This paper assesses each factor with data systematically collected from 8 stations over periods of 3 to 10 yr. To test the hypothesis that fluctuations in metal concentrations are affected by seasonal cycles in tissue

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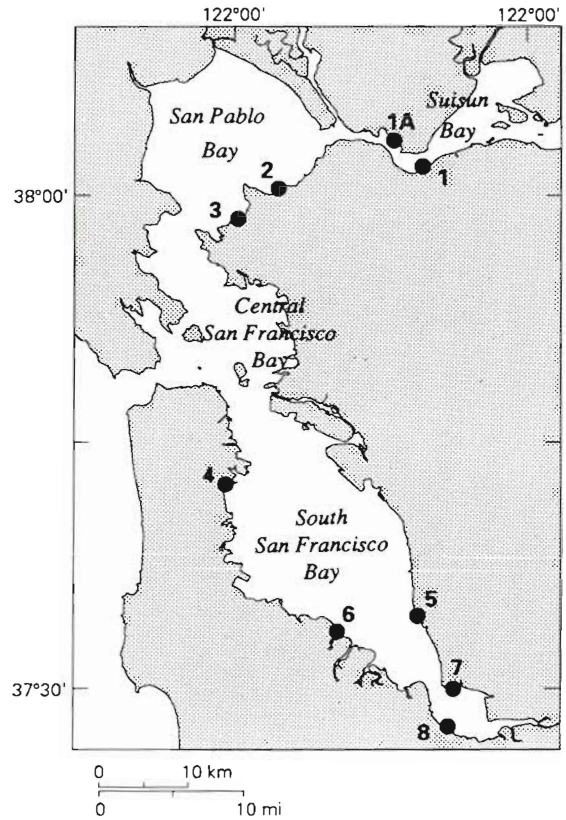

Fig. 1 San Francisco Bay showing location of stations

weight, Cain & Luoma's (1986) procedure is used to estimate changes in metal concentrations from changes in tissue weight during each sample interval. Estimated concentrations are correlated with observed concentrations at each station. Others have suggested that variations in metal content (μg $ind.^{-1}$) reflect changes in environmental exposure more reliably than metal concentrations (Simpson 1979, Fischer 1983, 1988). To test this, we compare temporal changes in metal content in *M. balthica* against inputs from a source of long-term metal discharge near one station, and compare temporal and spatial variation in content against sediment metal concentrations at all stations.

Metal content is dependent upon the size and age of the animal (Boyden 1974, Williamson 1979, Møhlenberg & Riisgård 1988). Our calculations of metal content in *Macoma balthica* are standardized to shell length. However, bivalves of similar shell length may differ in age from year-to-year or station-to-station due to variations in growth rate (Beukema et al. 1977, Wenne 1985, Wenne & Klusck 1985, Page & Hubbard 1987, Thompson & Nichols 1988). Thus, assessments of metal contamination may be biased by comparisons of metal content among animals standardized only to size (Williamson 1979, Cain et al. 1987). Data from earlier growth experiments (Thompson & Nichols 1988) are used to determine if differences in the age of *M. balthica* standardized to shell length affect comparisons of metal content across a contamination gradient.

Relations between metal content and sediment concentrations are employed to assess influences of hydrologic processes on metal bioavailability in San Francisco Bay. Copper and silver were studied because of their strong toxicity to aquatic organisms and their importance as pollutants to the San Francisco Bay system (Luoma & Phillips 1988).

MATERIALS AND METHODS

Field collection and analytical methods. Sediments from the oxidized, surficial layer, and 20 to 30 *Macoma balthica*, were collected at near-monthly intervals from 8 intertidal mudflats in San Francisco Bay (Fig. 1). These stations were selected to represent the range of geochemical and hydrologic conditions in the Bay (Luoma et al. 1985, Thomson-Becker & Luoma 1985). Stn 8 has been sampled continually since 1977; the other stations were sampled between 1977 and 1980. Clam tissues were prepared by methods previously described (Luoma & Cain 1979, Cain & Luoma 1986) and analysed for Cu and Ag by flame atomic absorption spectroscopy (AAS). Oxidized surficial sediments were prepared and analysed following procedures described by Thomson-Becker & Luoma (1985). Sediment was either extracted immediately and analysed for Cu by flame AAS, or dried to constant weight at 100 °C and stored. Dried sediment from Stns 1, 5 and 8 was later extracted with 0.6 N HCl and analysed for Ag by flame and furnace AAS (by method of standard additions). Data for metal discharge from a municipal sewage treatment plant near Stn 8 was provided by plant personnel.

The analytical quality was assured by processing certified standards (US National Bureau of Standards SRM 1566, oyster tissue, and SRM 1646, estuarine sediment). The mean concentrations (μg g^{-1}) of analysed standards typically were within 1 standard error of the certified mean concentrations. Ag concentrations of analysed SRM 1566 were slightly higher (1.05 ± 0.06) than the certified mean (0.89 ± 0.09).

Allometric model and calculations of metal content and concentration. The soft tissue weight of a clam of standard shell length (e.g. 25 mm) was followed over time by fitting data for each sample collected to the allometric equation

$$W = aL^b \quad (1)$$

where W = dry weight (mg); L = shell length (mm); and a and b = fitted parameters that change with the time of collection (Gallucci & Hylleberg 1976, Nichols & Thompson 1982, Cain & Luoma 1986).

The terms 'content' and 'body burden' are often used interchangeably with concentration in the literature. In this paper, we define content and body burden as the total mass of a metal in the body of a standard-length clam (µg ind.$^{-1}$). Total metal content, $\hat{M}_j$ (µg), was calculated for a clam of standard shell length (25 mm except where stated) by the following formula

$$\hat{M}_j = \left(\frac{\overline{M}}{W}\right)_j \hat{W}_j \qquad (2)$$

where $\left(\frac{\overline{M}}{W}\right)_j$ = mean metal concentration of the sample (µg g^{-1}) collected at time j; and $\hat{W}_j$ = dry tissue weight of the standard-length clam predicted from Eq. (1). If metal concentration correlated significantly ($p \leq 0.01$) with shell length within a sample, the concentration of a standard-sized clam, predicted from the correlation, was used instead of the mean metal concentration, $\left(\frac{\overline{M}}{W}\right)_j$, in Eq. (2). The standard deviation of collected samples typically ranged from 20 to 60 % of the mean metal concentration, $\left(\frac{\overline{M}}{W}\right)_j$, but occasionally was as great as 100 %.

The metal concentration expected solely from a change in tissue weight between consecutive samples (Cain & Luoma 1986) was calculated as

$$\left(\frac{\hat{M}}{W}\right)_{j+1} = \left(\frac{\hat{M}_j}{\hat{W}_{j+1}}\right) \qquad (3)$$

where $\left(\frac{\hat{M}}{W}\right)_{j+1}$ = calculated concentration at the time of sampling, j+1; and $\hat{W}_{j+1}$ = predicted weight of soft tissues at time j+1 (Eq. 1). Metal concentrations of tissues change reciprocally in proportion to changes in tissue weight if metal body burden ($\hat{M}_j$) remains constant (Cain & Luoma 1986). In this paper, the value of ($\hat{M}_j$) was continually adjusted to the observed value for each monthly sample and then $\left(\frac{\hat{M}}{W}\right)_{j+1}$ was recalculated for the following month. Calculated and observed concentrations at j+1 were then compared permitting a month-to-month analysis of the effect of weight change on tissue concentrations. This approach limits error propagation to a single month.

A critical element of the model calculations of concentration is the assumption that metal content is not affected by the 2 to 5-fold variation in tissue weight that characterizes the yearly growth cycle of *Macoma balthica* (Nichols & Thompson 1982, Cain & Luoma 1986, Thompson & Nichols 1988). This assumption is supported by the observations of others (Phillips 1976a, Simpson 1979, Fischer 1988) and studies showing that much of the annual fluctuation in tissue mass in *M. balthica* is from the accumulation and subsequent utilization of glycogen and lipids (Beukema & de Bruin 1977, Wenne & Styczynska-Jurewicz 1985, Jarzebski et al. 1986b), macromolecules which should be relatively nonreactive with metals. Regression-correlation analysis of Cu and Ag content in standard length *M. balthica* with seasonal changes in dry tissue weight also was insignificant (Cain & Luoma unpubl.).

Effects of age and size on metal content. Ages of *Macoma balthica* were determined from studies of shell growth at Stns 1A (data at this station also were used to estimate age at Stn 1), 5, and 8 (Thompson & Nichols 1988). Thompson & Nichols (1988) recorded incremental increases in shell length at monthly intervals between January 1983 and December 1984 in situ by release-recapture methods. From these data, cumulative shell growth was projected to estimate the shell length of a 4-yr-old clam at each station (Cain et al. 1987). Growth in 1982 and 1985 was estimated from Thompson & Nichols' (1988) data for 1983 and 1984, respectively, based upon the similarity in food availability (phytoplankton abundance) among these years (Alpine et al. 1985, 1988, Thompson & Nichols 1988). The age estimate was then applied to periods when metal data were available. Correlations with metal concentrations in sediment from Stns 1, 5, and 8 were compared before and after normalizing content for age.

RESULTS AND DISCUSSION

Seasonal fluctuations in metal concentrations

Concentrations of Cu and Ag in *Macoma balthica* showed a seasonal pattern of variation at most stations, especially Stn 8, similar to that predicted by the allometric model (Figs. 2 and 3). For example, predicted and observed concentrations at Stn 8 varied by as much as a factor of 5 and were highest during November to March and lowest during May to September. However, only 5 of 16 correlations of observed metal concentrations in *M. balthica* with those predicted from Eq. (3) were statistically significant ($p < 0.05$). At 5 of the 8 stations, less than 25 % of the observed variation in metal concentrations could be accounted for by changes in the weight of the clam (Table 1). At Stn 8, where the data set was most extensive, 67 % of the variation in Cu and 56 % of the variation in Ag could be explained statistically by seasonal changes in weight between 1977 and 1986. The fit between observed and predicted metal concentrations at Stn 8 differed among years. In 1980, only 1 % and 9 % of the variation in Cu and Ag, respectively, occurred coincident with weight change (Fig. 2). That year contrasted to 1979, when the pre-

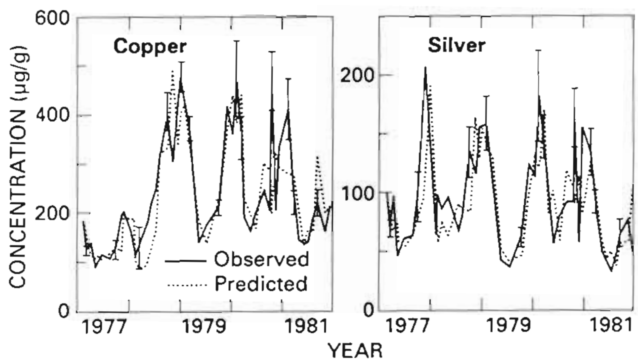

Fig. 2. *Macoma balthica*. Mean metal concentrations (solid line; representative error bars are ± 1 SEM) at Stn 8 plotted with concentrations predicted from changes in the weight of soft tissues (broken line). Data standardized to 25 mm shell length

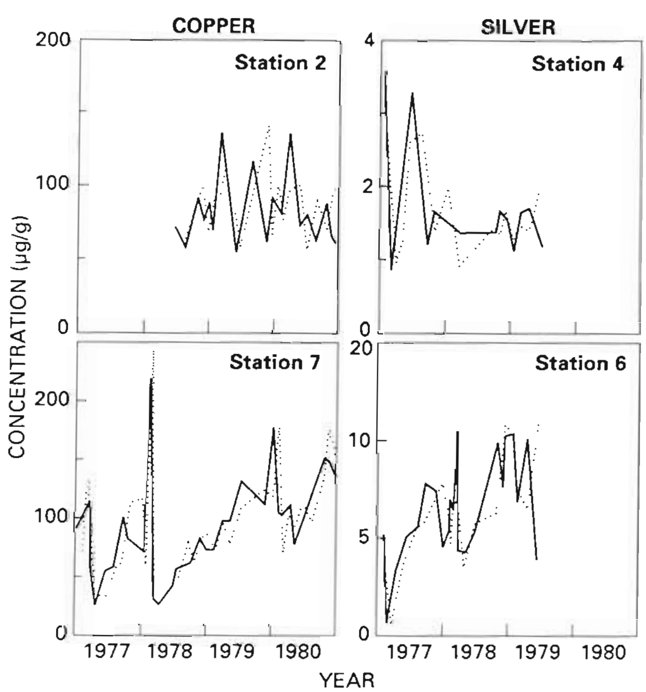

Fig. 3. *Macoma balthica*. Mean metal concentrations at Stns 2, 4, 6, and 7 (solid line) plotted with concentrations predicted from changes in the weight of soft tissues (broken line). Data standardized to 25 mm shell length except Stn 2 (20 mm)

dicted concentrations accounted for 78 % of the variation in Cu and 68 % of the variation in Ag.

Examination from month to month indicated that at specific times seasonal weight change could be an important factor affecting metal concentrations at stations even when overall correlations between observed and predicted concentrations were weak (Table 1). For example, the declines in metal concentrations in spring 1979 at Stn 2, and in spring 1977 at Stn 4, coincided

Table 1. *Macoma balthica*. Coefficient of determination, r^2, for the correlation of observed and predicted metal concentrations for standard-length (25 mm except Stn 2) clams from San Francisco Bay

Station	Metal	
	Cu	Ag
1	0.462***	0.169
2[a]	0.001	0.013
3	0.015	0.044
4	0.191	0.072
5	0.108	0.122
6	0.176	0.326**
7	0.224**	0.068
8	0.671***	0.565***

[a] Data standardized to 20 mm shell length; see 'Methods'
** $p < 0.01$
*** $p < 0.001$

with the decreases in concentrations predicted by the model from tissue growth (Fig. 3). Weight loss during August to February coincided with increases in metal concentrations in 1977 at Stn 6, and in 1978 at Stn 7 (Fig. 3). The best example occurred at Stn 7. Cu concentrations of *Macoma balthica* increased without seasonal fluctuation throughout 1978 and 1979 as tissue weight steadily decreased (Fig. 3). The unusual absence of tissue growth in older clams in the spring of 1979 and an apparent poor recruitment of juvenile *M. balthica* that year indicated that conditions at this station were unfavorable for normal growth. Thus, it appeared that Cu concentrations increased as the condition of the clams deteriorated.

Seasonality in metal content

A feature of the allometric model used in the study was that observed metal concentrations and those predicted from weight changes differed when metal content of the soft tissues changed during the sampling interval. At Stns 1 to 7, these changes in metal content exhibited few consistent intra-annual patterns from year-to-year; nor were patterns of fluctuation strongly consistent among stations (Figs. 4 and 5).

In contrast to Stns 1 to 7, some seasonality in metal content was evident at Stn 8. Peaks in Cu and, especially, Ag content were observed between November and April, while the lowest values typically occurred between May and October (Figs. 6b and 7b). The amplitudes of these seasonal changes, however, were less than differences between some years (e.g. between 1977 and 1981), and often were less than aperiodic episodes of fluctuation that also occurred within the time series.

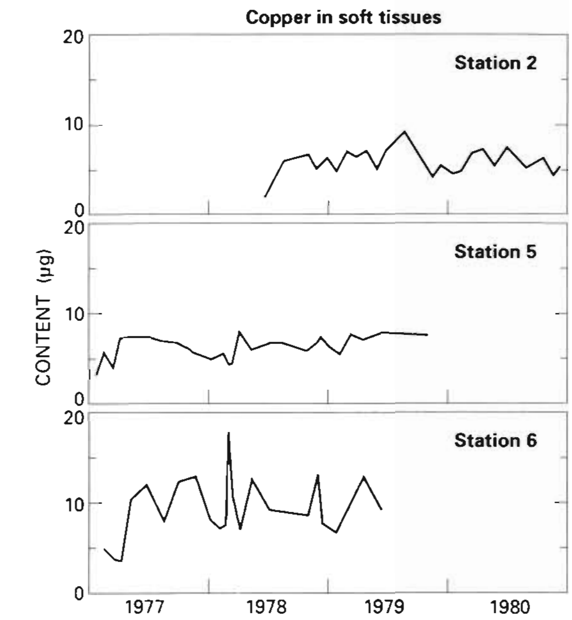

Fig. 4. *Macoma balthica.* Cu content of soft tissues at Stns 2, 5, and 6 during 1977 to 1980. Data standardized to 25 mm shell length except Stn 2 (20 mm)

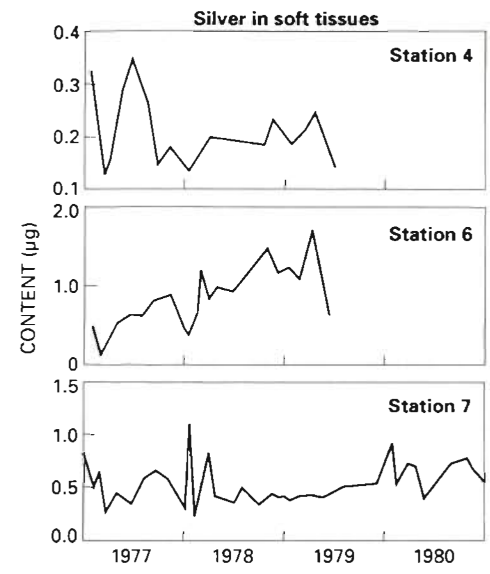

Fig. 5. *Macoma balthica.* Ag content of soft tissues at Stns 4, 6, and 7 during 1977 to 1980. Data standardized to 25 mm shell length

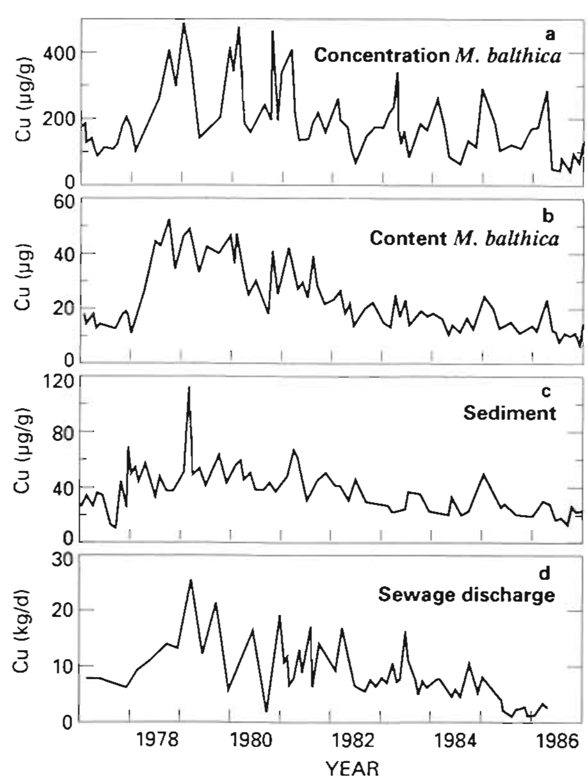

Fig. 6. Cu at Stn 8 during 1977 to 1986. (a) Concentration in soft tissues of *Macoma balthica;* (b) content in soft tissues of *M. balthica* (25 mm shell length); (c) concentration of surficial sediments; (d) rate of Cu discharged in sewage effluent

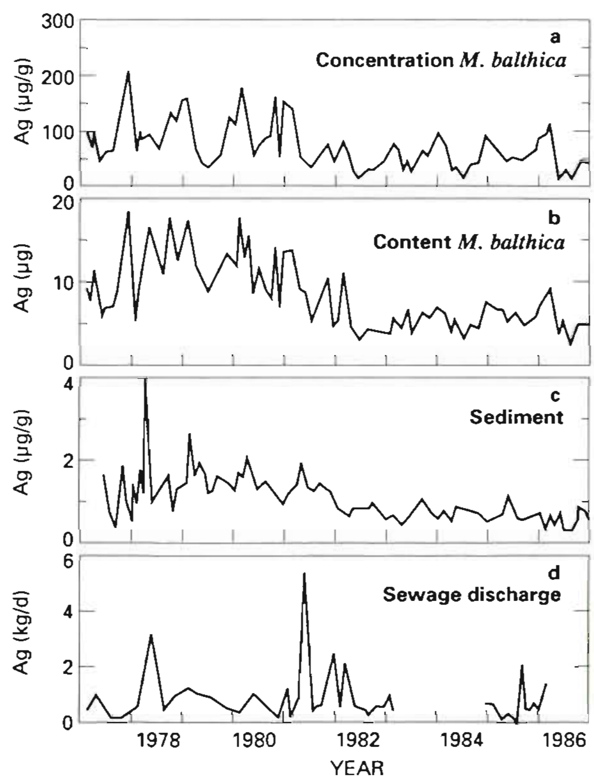

Fig. 7. Ag at Stn 8 during 1977 to 1986. (a) Concentration in soft tissues of *Macoma balthica;* (b) content in soft tissues of *M. balthica* (25 mm shell length); (c) concentration of surficial sediments; (d) rate of Ag discharged in sewage effluent

Clams with the highest levels of metal enrichment generally displayed the greatest variation in metal concentration. This observation appeared to be explained by the interaction between differences in metal body burden and changes in tissue weight. Changes in tissue metal concentrations for a given change in tissue weight were proportionate to metal content, and were accentuated when metal contents were elevated. For example, Cu and Ag content at Stn 8 tripled during 1978. As metal content increased, the change in concentration calculated for a change in tissue weight also increased, resulting in concentrations fluctuating by 400 $\mu g\ g^{-1}$ for Cu and 100 $\mu g\ g^{-1}$ for Ag during the year (Figs. 6a and 7a). In 1984, when metal contents were lower, Cu and Ag concentrations fluctuated by 200 and 75 $\mu g\ g^{-1}$, respectively, even though growth during this year was similar to 1978.

Effects of spawning on metal content of soft tissues reported for some bivalves (Zaroogian & Johnson 1983) were not readily apparent in this study. *Macoma balthica* in San Francisco Bay spawn primarily in January and February (Nichols & Thompson 1982), but tissue weight or metal content of mature individuals (> 20 mm shell length) during these months did not display any consistent change among years at any station. The increase in body weight during the spring reflects growth of non-reproductive tissues (Nichols & Thompson 1982, Wenne 1985, Jarzebski et al. 1986a). Lobel & Wright (1982c) also have related the seasonal dynamics of Zn in tissues of *Mytilus edulis* to fluctuations in the weight of somatic rather than gonadal tissues.

Relation of metal content to exposure

Fluctuations in metal content in *Macoma balthica* were significantly correlated to environmental exposures of Cu and Ag. A sewage treatment plant near Stn 8 has been identified as the source of Cu and Ag input to this station (Thomson et al. 1984). Long-term trends in metal discharge from the plant and metal concentrations in sediments are shown in Figs. 6 and 7. The Cu and Ag content of 25 mm *M. balthica* increased 5 and 3-fold, respectively, in 1978–1979 at Stn 8, coincident with increased discharge of metals from the treatment plant and increases in metal concentrations of sediments (Figs. 6 and 7). Metal content in *M. balthica* at Stn 8 began to decrease in 1981 following the initiation of improved treatment processes at the plant, and in 1986 was back to levels first observed in 1977 (Fig. 6). The average Cu content in *M. balthica* for each year was highly correlated with yearly average Cu discharge (r^2 = 0.837; $p < 0.001$). Ag in *M. balthica* also decreased between 1980 and 1982 and after 1983 appeared to stabilize at levels similar to 1977 (Fig. 7). The record of Ag in effluent, however, was erratic and incomplete, and did not clearly reflect the decrease in Ag observed in sediments and *M. balthica*.

The long-term trends in metal concentrations in sediments at Stn 8 more closely coincided with metal content of *Macoma balthica* than with the complex trends in tissue metal concentrations. The coefficients of determination, r^2, for the correlations of log Cu sediment concentration with log Cu content and log Cu concentration of *M. balthica* were 0.456 and 0.207, respectively. Correlations of log Ag sediment concentration also were stronger with log Ag content of the clam (r^2 = 0.419) than with the log Ag concentration of the clam (r^2 = 0.166). The highly significant correlations ($p < 0.001$) with environmental indicators of metal exposure strongly supports the value of metal content as an indicator of biologically available metal (Simpson 1979).

Metal content also was more sensitive than metal concentration to transient changes in Cu and Ag exposure. For example, sediment Cu concentrations at Stn 8 exceeded 100 $\mu g\ g^{-1}$ for a brief period in the spring of 1979, the highest concentration observed at this station (Fig. 6c). A peak in Cu content was observed at the same time (Fig. 6b). Similarly, an increase in Ag content was observed in the spring of 1978 following a sharp peak in sediment Ag concentration (Fig. 7b, c). Neither of these events was clearly reflected by the metal concentration of the tissues because both occurred during periods of rapid tissue growth.

At other stations, temporal trends in metal content of *Macoma balthica* generally reflected temporal trends in sediment metal concentrations (Figs. 8 and 9), but correlations were insignificant. Small ranges in sediment metal concentrations (Luoma 1983), the insensitivity of whole body metal burdens to small episodic changes in metal bioavailability (Johannson et al. 1986), lags in the changes in metal content after changes in exposure, exposure to sources not detected by sediment analysis, and the shorter sampling period (3 to 4 yr) may have contributed to the weak relations within each station.

Across the broader range of sediment concentrations represented by the monthly data from all 8 stations, metal content of *Macoma balthica* correlated strongly with sediment metal concentrations (r^2 = 0.329 and 0.670 for Cu and Ag, respectively; $p < 0.001$). These correlations were not greatly different than correlations of metal concentration of *M. balthica* and sediment metal concentrations (r^2 = 0.342 and 0.618 for Cu and Ag, respectively; $p < 0.001$). These results indicate that metal content and concentration of soft tissues may yield similar indications of exposures, provided seasonal biases in concentration data are averaged by frequent sampling throughout a year.

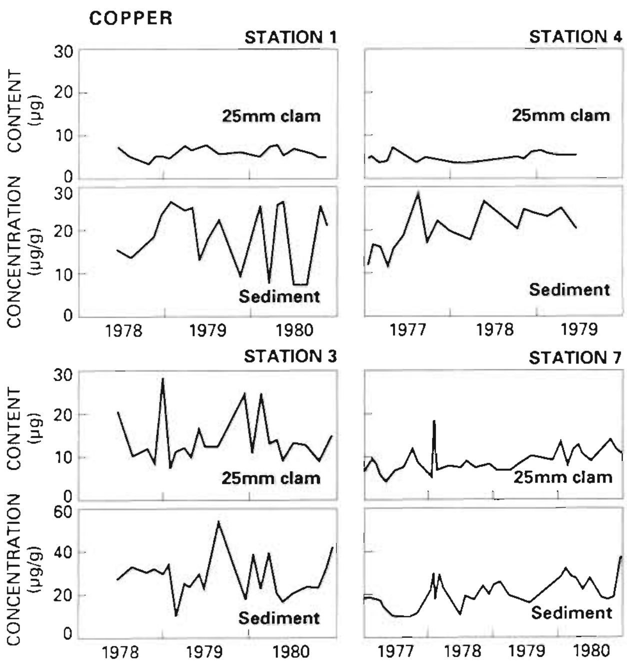

Fig. 8. Cu in *Macoma balthica* (upper panels) and surficial sediments (lower panels) at Stns 1, 3, 4, and 7 during 1977 to 1980

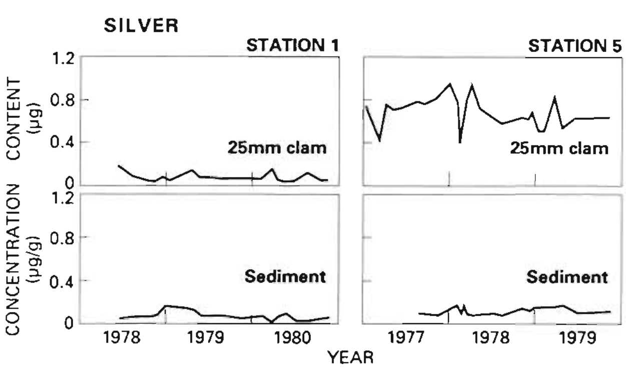

Fig. 9. Ag in *Macoma balthica* (upper panels) and surficial sediments (lower panels) at Stns 1 and 5 during 1977 to 1980

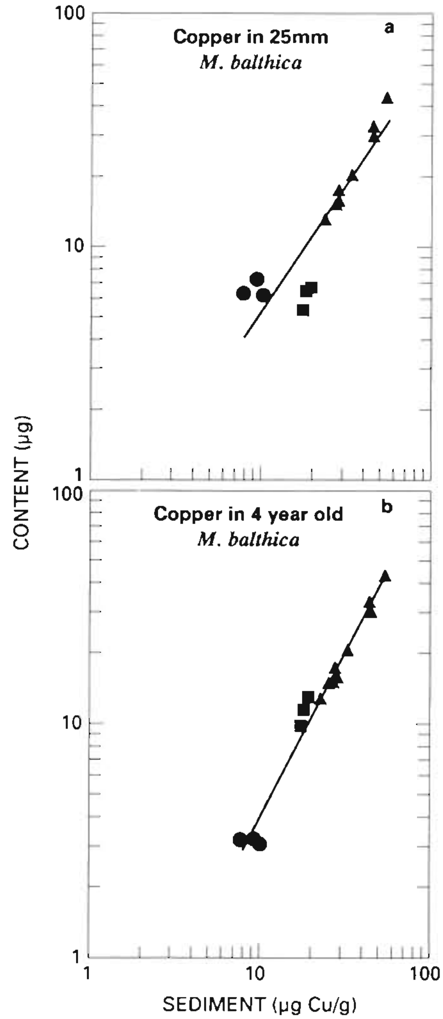

Fig. 10. Relation of sediment Cu concentration and Cu content of *Macoma balthica* at Stns 1 (■), 5 (●) and 8 (▲). Values are the yearly means of near-monthly samples. (a) Data for 25 mm clams; (b) data for 4-yr-old clams

Age-dependent effects on estimates of size-standardized metal content

Differences in the ages of clams of standard shell length appeared to slightly bias the relations between metal content and metal concentrations in sediments. Studies of shell growth (Thompson & Nichols 1988) showed that the shell lengths of clams of similar age consistently differed by 5 to 10 mm between North Bay (Stn 1A) and South Bay locations (Stns 5 and 8) (Cain et al. 1987). Values of metal content at Stns 1, 5, and 8 were affected by whether content was based upon the same shell length or the same age. Annual mean Cu content standardized to shell length correlated significantly with sediment concentrations, but the coefficient of determination was improved by 16 % when the correlation was done with age-standardized content (Fig. 10). The improvement resulted from a better fit of the data from the less enriched stations, 1 and 5, to the log-log regression. When Ag content was standardized to age, rather than size, the improvement in the correlation was smaller than Cu, because Ag contents at Stns 1 and 5 were only 1 to 16 % those at Stn 8. These results suggest that metal content in *Macoma balthica* was related more to age than shell length (size) as has been suggested for other organisms by other authors (Williamson 1979, Møhlenberg & Riisgård 1988). Samples from uncontaminated stations (e.g. Ag at Stns 1 and 5) may be the least susceptible to age-related biases because metals are accumulated by clams more slowly with age there than in contaminated areas. In general, considerations of animal age in the calculation of metal content may improve the sensitivity in comparisons of biologically available metal among stations.

Determining the age of bivalves is not a trival exercise. Release-recapture methods or analysis of shell rings or size-frequency histograms have inherent errors, are difficult to interpret, and are impractical for most routine environmental monitoring (Berta 1978, Nichols & Thompson 1982). Allometric ratios of shell size may be an alternative method (Seed 1980, Lobel & Wright 1982a,b, Fischer 1983). The ratio of shell weight to shell length appeared to be a useful index of relative age of *Macoma balthica* in San Francisco Bay (Cain unpubl.). Initial results of the analysis of samples collected during May to July 1987 at Stns 1, 5 and 8 showed that the slope of the regression of shell weight on shell length was roughly 3 for all stations, with slight variations from month to month at each station. Analysis of covariance further showed that the coefficient at Stn 1 was significantly smaller ($p \leq 0.05$) than those at Stns 5 and 8, indicating that growth rates were faster at Stn 1 than at the South Bay stations. However, in view of the phenotypic plasticity that can be expressed in the size and shape of the shell (Seed 1980), the value of this index cannot be assessed without more study.

Spatial and temporal distributions of bioavailable Cu and Ag

If spatial and temporal variations in metal content generally coincide with indicators of environmental exposures where the latter are known, then inferences about exposure can be made from content data where exposures are not known. Spatial distributions of bioavailable Cu appeared to be characterized by small-scale (station by station), rather than regional, variability (Fig. 11), as suggested in earlier studies (Girvin et al. 1975, Bradford & Luoma 1980, Goldberg et al. 1983, Smith et al. 1986). In contrast, the availability of Ag was distinctly different between North and South Bay. With the exception of Stn 4, Ag contents in South Bay were at least 3 times greater than in North Bay (Fig. 11), with the highest Ag body burdens in clams at Stn 8 in the extreme South Bay. Even after a significant decline in the 1980's, Ag content at Stn 8 in 1986 remained 5 to 40 times greater than at other stations in our study. Other localized areas of severe Ag contamination have been identified in South Bay (Girvin et al. 1975, Risebrough et al. 1978, Thomson et al. 1984, Smith et al. 1986); however, the individual and cumulative effect of such 'hot spots' on regional patterns of contamination is unknown (Luoma & Phillips 1988).

Increases in metal content in *Macoma balthica* were evident at several South Bay stations between 1979 and 1980, and no sustained decreases in content were detectable at any station during this time. Copper at Stn 7 (Fig 8) and Ag at Stn 6 (Fig. 5) increased significantly during 1977 to 1980 ($p \leq 0.05$; ANOVA). The increase in Ag content is consistent with other data that indicate Ag contamination in this region of the Bay increased sometime between the early 1970's and the late 1980's (Luoma & Phillips 1988). Such results suggest that the increase in metal discharge documented at Stn 8 between 1978 and 1981 may also have occurred at other localities.

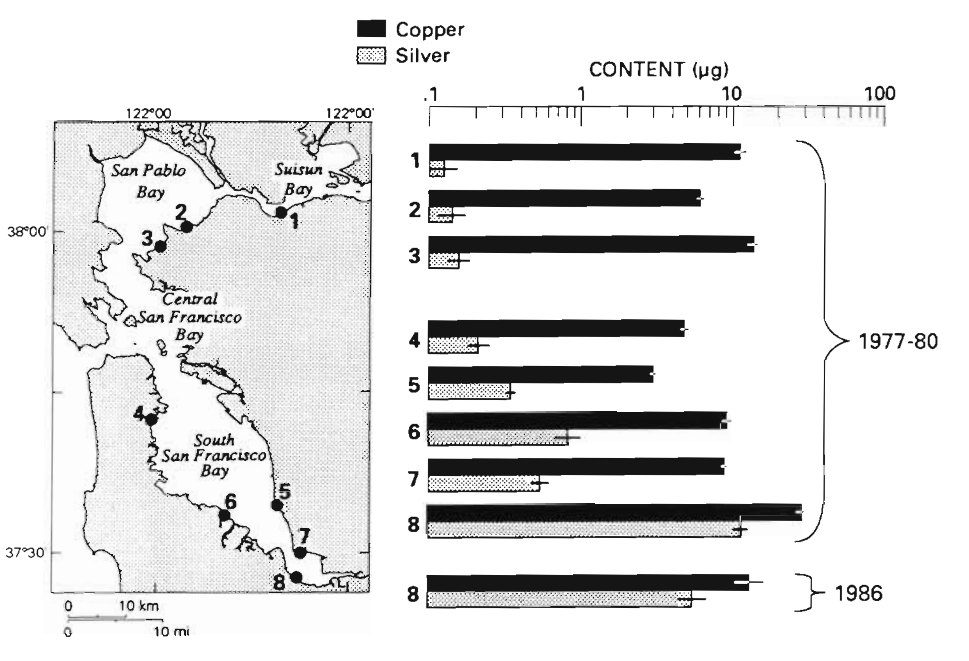

Fig. 11. *Macoma balthica.* Cu and Ag content in the soft tissues of standard-length clams in San Francisco Bay. Values are the means and 95% confidence intervals for near-monthly samples for the period 1977 to 1980. Content for 1986 at Stn 8 also shown. Data are standardized to 25 mm shell length except Stns 1 (30 mm), 2 (20 mm) and 5 (20 mm)

Factors affecting metal availability in San Francisco Bay

Although other authors have suggested that runoff and salinity are important forces driving changes in biologically available metals (Phillips 1976b, Cossa & Rondeau 1985, Nugegoda & Rainbow 1989) neither of these factors appeared clearly related to fluctuations in Cu and Ag content of *Macoma balthica* in San Francisco Bay. No consistent increases in Cu and Ag content in *M. balthica* occurred at Stns 1 to 7 during the distinct November to March increase in urban runoff characteristic of this estuary; nor did variations in Cu and Ag content within years show any consistently significant correlation with salinity at Stn 8 (Cain & Luoma unpubl.). Interaction with other factors may make any effects of runoff or salinity difficult to detect.

The winter maximum/summer minimum in metal content of *Macoma balthica* at Stn 8 may have been influenced by freshwater discharge, even in the absence of a direct salinity effect. Longer residence times of waters in the extreme South Bay during periods of low freshwater discharge (Conomos 1979) could allow contaminants from local sources to accumulate (Luoma & Cain 1979, Luoma et al. 1985, Nichols et al. 1986). Freshwater inputs also are accompanied by complex changes in sediment geochemistry that are out of phase with changes in salinity (Thomson-Becker & Luoma 1985). These could affect metal bioavailability (Luoma 1983, 1989).

CONCLUSIONS

Specific processes controlling variation of metals in organisms, in general, are difficult to conclusively demonstrate in nature because of the complex interaction of uncontrolled and unknown variables. However, there is sufficient understanding, from experimental studies, of processes affecting metal dynamics in organisms to begin to apply against field-collected data. The approach taken in this study considered empirical evidence to define how 3 factors affect metals in bivalves: (1) effects of annual growth cycles on tissue metal concentrations, (2) the effect of contamination on tissue metal content, and (3) age dependency of metal accumulation. The influence of these different factors was tested by correlation against observed data encompassing a wide range of geochemical, hydrological, and biological conditions. Although the correlative relationships are not direct evidence for causitive mechanisms, they suggest a hypothetical framework from which environmental and biological factors affecting tissue metal levels in *Macoma balthica* may be evaluated. The following conclusions are made with reference to *M. balthica*, but may be applicable to other species used for environmental assessment.

(1) The interpretation of data from biological indicators should include considerations of the species' life history, as biological processes may be dominant factors influencing metal dynamics in the organism. Metal concentrations in *Macoma balthica* frequently were lowest between May and September and highest between November and March apparently because of the alternation in tissue weight that characterizes the species' yearly growth cycle. Interpretations of such temporally variable concentrations could be biased by the time of year collections are made. Comparisons among populations also could be compromised if rates of growth or synchronism of the growth cycle differed.

(2) The Cu and Ag content of standard length clams did not display a consistent seasonal pattern of variation, but generally followed chemical indicators of environmental exposure in a 10-yr set of data collected at one station, and when all data from all stations were considered. This analysis provides evidence of the value of metal content as an indicator of biologically available metal.

(3) Differences in the ages of standard length clams collected from different stations appeared to bias spatial assessments of environmental metal contamination. Whenever possible, relations between age and metal content should be considered along with size.

(4) Long-term studies (5 to 10 yr) can be invaluable in assessing processes affecting the fate of biologically available metal in estuaries. Seasonal and year-to-year trends are more difficult to discern over shorter time scales, although the sensitivity of interpretations is greatly improved if data are collected at frequent (e.g. monthly or bimonthly) intervals. Determinations of near-monthly metal content in *Macoma balthica* revealed individual patterns of temporal fluctuation in metal content within and between years among stations in the Bay, with episodes of metal accumulation and loss occurring at most stations. The periodicity of these episodes ranged from years (e.g. Stn 8) to 1 mo. Cu and Ag contamination appeared to increase at several localities in San Francisco Bay between 1977 and 1980. None of the 8 stations studied showed a decline in either of these 2 important contaminants during this time. More recent data indicated that investment in improved waste-water treatment reversed the increasing trend at one locality. However, contamination is currently at a level that was characteristic of 1977, and remains especially substantial for Ag.

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