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Assessing Toxicant Effects in a Complex Estuary: A Case Study of Effects of Silver on Reproduction in the Bivalve, *Potamocorbula amurensis*, in San Francisco Bay

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ABSTRACT

Contaminant exposures in natural systems can be highly variable. This variability is superimposed upon cyclic variability in biological processes. Together, these factors can confound determination of contaminant effects. Long term, multidisciplined studies with high frequency sampling can be effective in overcoming such obstacles. While studying trace metal contamination in the tissues of the clam, *Potamocorbula amurensis*, in the northern reach of San Francisco Bay, an episode of high Ag concentrations was identified (maximum of 5.5 $\mu g\ g^{-1}$) at two mid-estuary sites. High concentrations were not seen in clams up-estuary (maximum of 1.92 $\mu g\ g^{-1}$) from these sites and were reduced down-estuary (maximum of 2.67 $\mu g\ g^{-1}$). Silver is not common naturally in the environment, so its elevated presence is usually indicative of anthropogenic influences such as municipal and industrial discharge. Monthly sampling of reproductive status of clams characterized the reproductive cycle and differences in the patterns of reproductive activity that corresponded to changes in Ag tissue concentrations. The proportion of reproductive clams was less than 60% during periods when tissue concentrations were high (generally >2 $\mu g\ g^{-1}$). When tissue concentrations of Ag decreased ($\leq 1\ \mu g\ g^{-1}$), the proportion of reproductive clams was 80 to 100%. A comparison between the annual proportion of reproductive clams and annual Ag tissue concentrations showed a significant negative correlation. No other measured environmental variables were correlated with reproductive impairment. The weight-of-evidence approach strongly supports a cause and effect relationship between Ag contamination and reduced reproductive activity in *P. amurensis*.

Key Words: silver, reproduction, bivalve, *Potamocorbula amurensis*, San Francisco Bay.

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INTRODUCTION

Ecosystems are complex and characterized by a high degree of environmental variability. Trying to distinguish cause-and-effect relationships amid this variability can be very challenging. However, the variability of some environmental parameters characteristic of ecosystems can have repeated and somewhat predictable patterns (*i.e.,* variability is dynamically stable or non-directional) (Luoma *et al.* 2001). Identification and appropriate sampling of those patterns can aid explanation of processes and can help in identifying effects of anthropogenic influences like pollutant inputs. While studying trace metal contamination in the tissues of the clam, *Potamocorbula amurensis*, in the northern reach of San Francisco Bay, an episode of high Ag concentrations in the clams at two mid-estuary sites was identified. No other metal measured in the clam (Cd, Cr, Cu, Ni, Pb, V, and Zn) showed a similar trend as seen with Ag. This resulted in further investigations into this unique observation. The objective of this paper is to describe the relationship among the Ag contamination in *Potamocorbula amurensis*, hydrodynamic influences in the northern reach of the bay, and changes in reproductive activity in the clam. Causality between Ag accumulation and its effect on the reproductive activity of the clam is discussed in the context of the seven weight-of-evidence criteria described in Adams (2003). From the patterns in the variability of the data, the environmental stress imposed by Ag is the most likely factor causing changes in the reproductive activity in the clam.

Silver is not common naturally in the environment, so its elevated presence in water, sediment or biological tissues is usually indicative of anthropogenic influences (Smith and Flegal 1993; Hornberger *et al.* 2000; Luoma and Phillips 1988). Silver is an important contaminant because of its high toxicity in estuarine waters. It is very particle-reactive and rapidly taken up by phytoplankton. Both of these processes may enhance its availability via diet (Luoma *et al.* 1995; Wang *et al.* 1995, 1996). Although the dissolved concentrations of silver that cause direct toxicity are orders of magnitude higher than typically occur in even contaminated settings, experiments show that low concentrations of silver in the food of marine animals (only 3 to 10 fold higher than natural background concentrations) can cause sublethal effects such as reproductive impairment (Hook and Fisher 2001).

It is well recognized that environmental variability can confound interpretation of potential influences of multiple stressors or the ability to separate out the influences of a single stressor from natural variability. Nevertheless, in some circumstances, identification of stressor effects can be distinguishable, although not necessarily simple; especially if interpretations follow structured assembly rules (see Adams 2003). One of those circumstances is recovery of a population from a pollutant effect in a field setting. The approach to separating complexities must involve long-term sampling, at an intensity consistent with the dynamic stabilities of the environment, as the system recovers (Hornberger *et al.* 2000). Numerous opportunities for such studies exist (or have existed) in the developed world since the 1970s, although few studies have appropriately exploited them. Hydrology, water chemistry, sediment characteristics, food availability, and other ecologically important processes vary widely from year-to-year in estuaries. Yet on the time scale of a decade or more, unidirectional trends in these variables are rare. If pollutant exposure is superimposed on this variability, its effects cannot be separated, convincingly, in a few years of sampling. However, if pollutant inputs are unidirectionally declining, as has happened often in the United States since the passage of the Clean Water Act in 1972, the downward trend in exposure may be the only decadal scale trend in the data. Hornberger *et al.* (2000) used such a trend (and a weight-of-evidence approach) to isolate apparent effects of silver and copper on reproduction in the bivalve *Macoma balthica* using more than 20 years of high-frequency data from a nearby municipal sewage treatment plant in south San Francisco Bay. In the present paper, we use a similar approach with 10 years of near monthly data to isolate what appear to be reproductive effects of Ag on the bivalve, *P. amurensis*, in a setting in north San Francisco Bay.

MATERIALS AND METHODS

P. amurensis was collected from four subtidal sites in the northern portion of San Francisco Bay at near-monthly intervals from 1990-1999 (Figure 1). The sites were located in the ship channel where depths ranged from 8 to 20 m and included Mallard Island (MI), Concord Naval Weapons Station (CNWS), Carquinez Strait (CS), and San Pablo Bay (SPB). The MI site is the most landward (closest to the Sacramento/San Joaquin Rivers) of the sites and SPB the most seaward (closest to the Pacific Ocean) site. Samples were collected with a 0.10 m^2 Van Veen grab and washed in a 4-mm screen sieve. Clams from each site (40 to 100 individuals) were depurated for 48 h in the ambient water from the grab, then separated into 3 to 14 individual size composites. The number of size composites depended upon the total number of clams collected at each site. Clams ranged in size from 8 to 25 mm. Each size composite contained 5 to 25 individual clams of similar shell length (within 1 mm). The number of clams included in each size composite was dependent on the number collected of each size and the size itself (*i.e.,* more 8 mm clams (n=25) were needed for adequate mass for an accurate analysis than the 20 mm clams (n=5)).

Silver concentrations ($\mu g\ g^{-1}$) were determined by the method described in Brown and Luoma (1995). Briefly, the soft tissue was removed from the shell and combined within each replicate size composite. The tissue was dried, digested by reflux in concentrated nitric acid (Luoma and Bryan 1981), and reconstituted in 5% hydrochloric acid, then analyzed by ICP-AES. Silver concentrations measured in *P. amurensis* after depuration reflect what was accumulated in the tissues, without influences from gut content (Brown and Luoma 1995). Internal standards and standard reference material (National Institute of Standards and Technology standard oyster tissue no. 1566a) were used to assure accuracy and precision of the data.

The collection and determination of reproductive stage is described in detail in Parchaso and Thompson (2002). Briefly, clams were collected for reproduction analysis concurrently with the clams collected for Ag analyses. The clams used for reproduction analysis were preserved in 10% buffered formalin after collection. In the laboratory, the soft tissue of each clam was removed and stored in 70% ETOH. The tissues were prepared using standard histological techniques. Each stained 10 μm thin section was examined with a light microscope and characterized by sex and developmental stage of the gonads, thus allowing each specimen to be placed in one of five qualitative stages of gonadal development: inactive, active, ripe, spawning, and spent. Frequently, however, individuals were in transition between stages (*i.e.,*

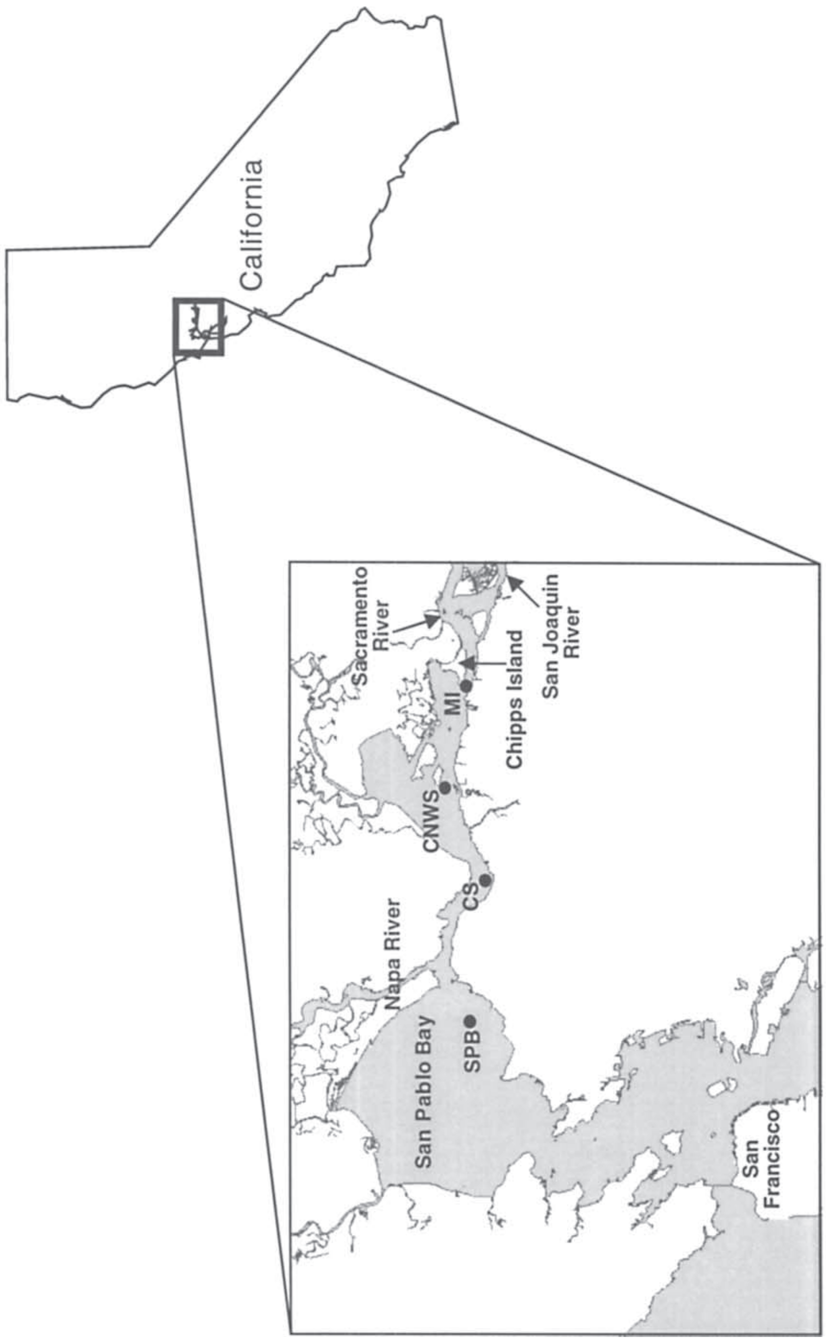

Figure 1. **Map of the northern portion of San Francisco Bay with site locations.** Site names are designated as: MI (Mallard Island); CNWS (Concord Naval Weapons Station); CS (Carquinez Strait); SPB (San Pablo Bay).

active to ripe). To alleviate the difficulty of determining the stage of these individuals, the percentages of clams in the active, ripe, and spawning stages were given positive values (multiplied by +1), while the percentages of clams in the inactive and spent stages were given negative values (multiplied by –1). These values were summed for each month at each station, and represent the "central tendency of reproduction". For example, a month with 30% active, 10% ripe, and 70% inactive would be scored as –30%. The central tendency gives an indication of the reproductive state for the population sampled at that time. Data for reproduction were available through 1997.

The hydrologic data are an estimate of net Delta outflow near Chipps Island (Figure 1) and were derived by performing a water balance about the boundary of the Sacramento–San Joaquin Delta, taking Chipps Island as the western limit. The data were provided by the Interagency Ecological Program (IEP) (2002).

RESULTS

Hydrologic Setting

The northern portion of San Francisco Bay (SFB) is influenced by large seasonal and year-to-year fluctuations in freshwater inflow from the Sacramento and San Joaquin rivers, which are the source of 90% of the freshwater inflow into the Bay (Figure 2) (Conomos *et al.* 1985). Each year is characterized by a distinct high and low inflow period driven by the Mediterranean climate of the region, snowmelt runoff, and controlled releases from the reservoirs. The magnitudes of these fluctuations differ among years. This study occurred over a period of extreme year-to-year differences in the hydrodynamics. Drought conditions occurred in 1989 through 1992, and 1994, when the annual mean inflow of freshwater from the rivers was 152 to 252 $m^3 s^{-1}$ (Figure 2). The wet season of 1993 marked the end of a 7-year drought with annual mean inflow of 760 $m^3 s^{-1}$. Annual mean inflow increased to a range of 1094 to 1823 $m^3 s^{-1}$ in 1995 to 1999. The seasonal (intra-annual) and yearly (inter-annual) variability in freshwater flow exposed the species living in this segment of SFB to changes in salinity, river-borne contaminant inputs, the distribution of sediments and contaminants within the bay, and the carbon load to the Bay (Nichols *et al.* 1986; Jassby *et al.* 1993). Although freshwater inflows were highly variable inter-annually, there was no simple unidirectional trend (*i.e.,* consistent increase or decrease over time) in the variability over the 10-year study period (Figure 2). In contrast to the extreme inter-annual differences, the intra-annual pattern in variability of river inflow, and the environmental factors related to river inflow, is somewhat predictable. River inflow is highest in the winter and lowest in the summer and fall. A relatively predictable spatial (landward-to-seaward) gradient in salinity reflects these influences, although the spatial range of the salinity gradient can vary from year to year (Conomos *et al.* 1985). For example, if the freshwater intrusion into the bay increases (travels more seaward), the saltwater intrusion into the bay decreases (does not extend as far landward). Understanding the patterns in both inter-annual and intra-annual variability is critical to understanding the differences between natural and anthropogenic effects on the ecosystem.

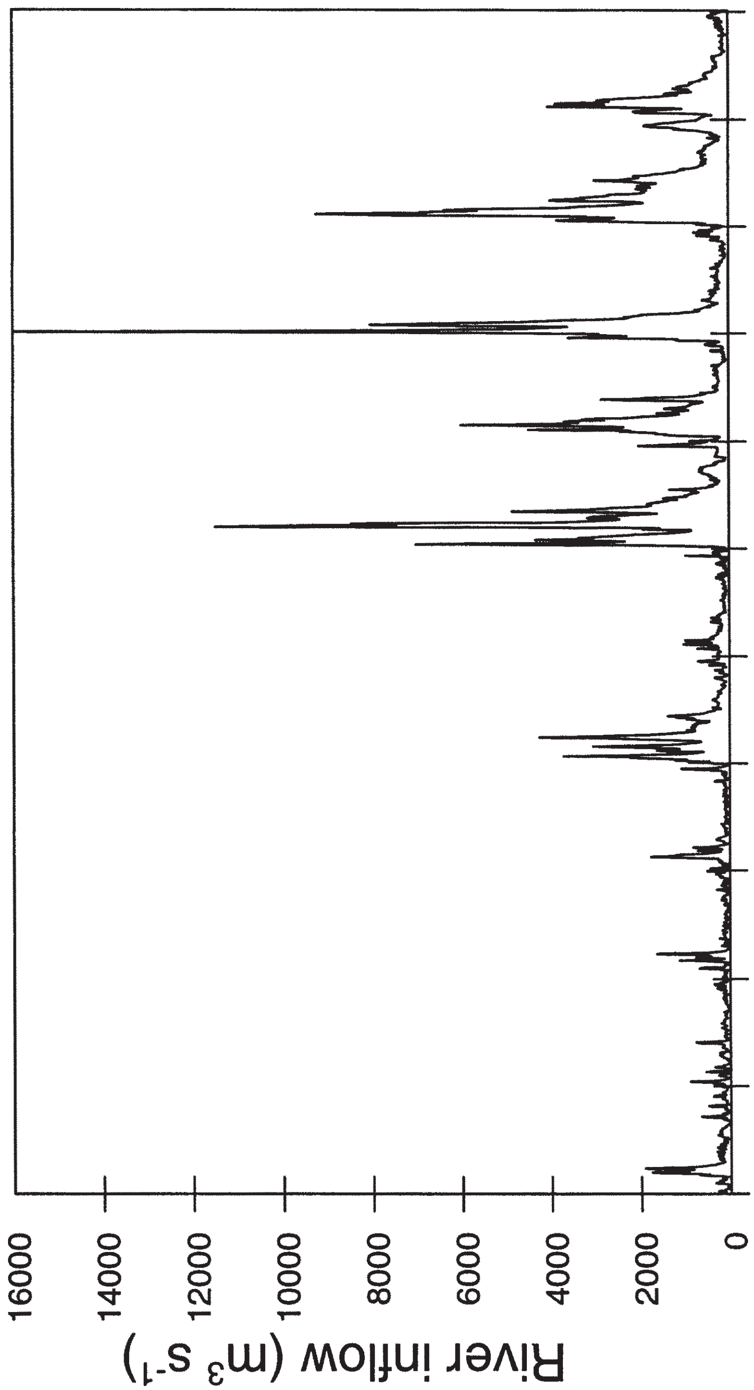

Figure 2. Hydrograph of daily river inflow data of freshwater from the Sacramento and San Joaquin rivers.

Silver in the Tissues of *Potamocorbula amurensis*

On all time scales (monthly, annually, and over the decade), the highest silver (Ag) tissue concentrations in *Potamocorbula amurensis* occurred in the populations at the two mid-estuary sites, Concord Naval Weapons Station (CNWS) and Carquinez Strait (CS). When data from all 10 years were aggregated, concentrations at CNWS and CS were significantly higher (twofold) ($p \leq 0.05$) than the two end-estuary sites at Mallard Island (MI) and San Pablo Bay (SPB) (Figure 3). This was largely because annual mean silver concentrations at the two mid-estuary sites (CNWS and CS) were higher than at the other sites in all years before 1996 (Figure 4). Annual mean concentrations decreased over time at CNWS and CS (means in the range of 2 to 4 $\mu g\ g^{-1}$; from1990-1993 at CNWS, and from 1990 to 1995 at CS). After 1995, annual mean concentrations at these two sites decreased until concentrations in the mid-estuary clams were as low as the concentrations in the clams at the two end-estuarine sites (mean of $\leq 1\ \mu g\ g^{-1}$, sites MI and SPB).

Monthly mean concentrations of silver in the tissues of *P. amurensis* at the two end-estuary sites (MI and SPB) were low during all seasons ($\leq 1\ \mu g\ g^{-1}$) for the majority of the study (Figure 5). A slight increase was observed at both sites in 1991-1992 when tissue concentrations increased to a maximum of 1.9 $\mu g\ g^{-1}$ at MI in 1991 and 2.7 $\mu g\ g^{-1}$ at SPB in 1992. Concentrations were more variable on a monthly scale at CS and CNWS than at MI and SPB, but, nevertheless, exposures were consistently greater than at the other two sites until the late 1990s, especially toward the end of the season of high inflow in each year. Thus at all time scales, the unidirectional downward trend in Ag accumulation at CS and CNWS was unambiguous over the 10 year period; and the differences in Ag accumulation between the CS and CNWS sites and the MI and SPB sites were significant at the beginning of the 1990s and converged as the decade ended.

Influence of River Inflow on Within Site Variability in Silver Tissue Concentrations

A comparison of the patterns in tissue concentrations of Ag and river inflow indicated that, within each site, the variability of silver concentrations was related to the pattern of freshwater inflow from the Sacramento and San Joaquin rivers (Figure 5); most noticeably at the sites of highest silver concentrations (CNWS and CS). For example, Ag concentrations in the clams at CS decreased as high (winter) river inflow began. During the period of low river inflow, concentrations in the tissues steadily increased until the next episode of high river inflow began. Deviations from this pattern occurred at CS only in very low flow years. For example, in 1993, tissue Ag concentrations declined after the winter rains, but steadily increased through 1994 (a critically dry year), to levels as high as those seen in 1990. After 1995, there were no periods of low flow that extended beyond the typical seasonal pattern, and Ag in the tissues did not accumulate as high as they had prior to 1995. This suggested that the longer the period of low flow, the greater the accumulation of Ag. The pattern was not as distinct at CNWS, but sampling was less frequent (bimonthly) and concentrations were higher than at CS in the early years, perhaps indicative of a local influence. At both sites, the maximum Ag tissue concentration observed during each period of extended low flow ($< 1500\ m^3\ sec^{-1}$) was significantly

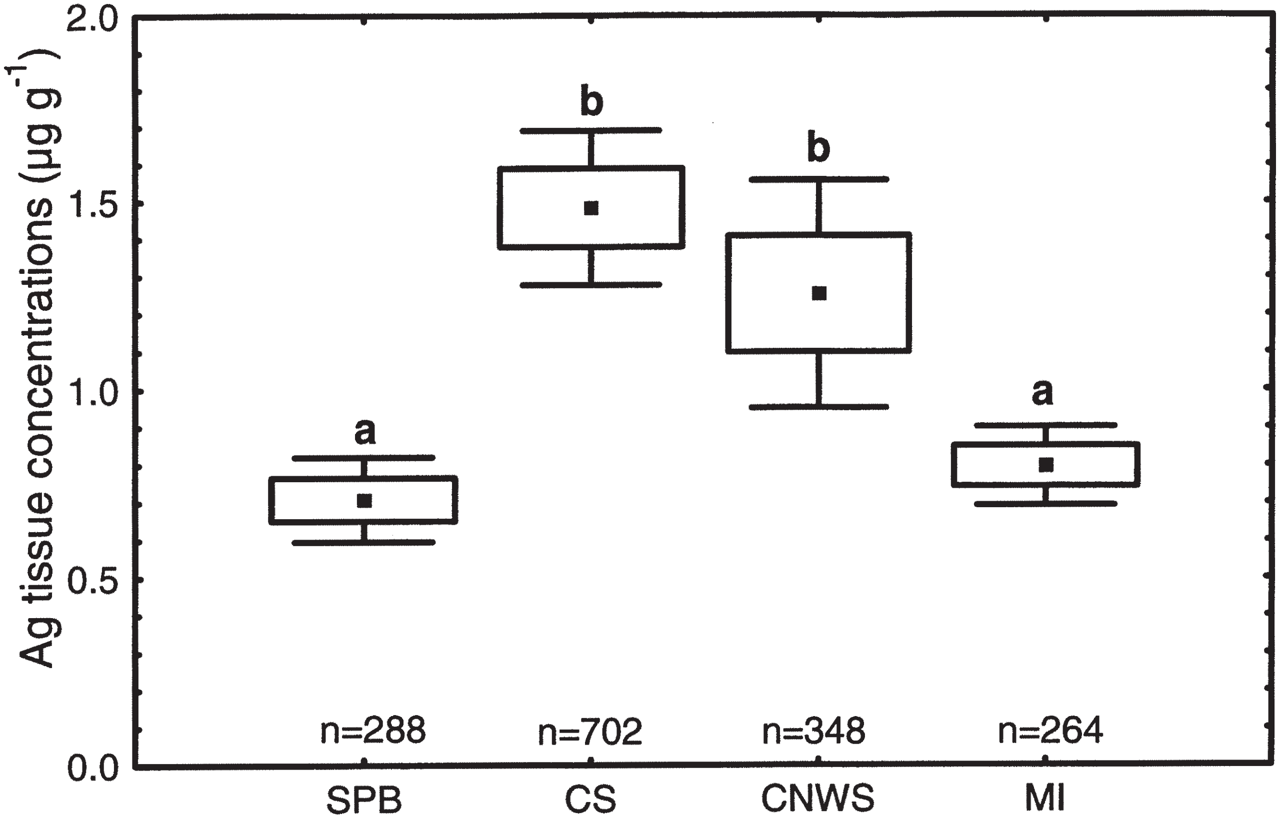

Figure 3. **Grand mean silver tissue concentrations in *Potamocorbula amurensis* at each site.** The points represent the grand means of all data from 1990 to 1999 in µg g^{-1} dry weight, with number of samples (n) per mean shown at the bottom of the graph. The boxes represent 1 standard error and the whiskers represent 1.96 standard error. The letters a and b designate significant differences among means ($p \leq 0.05$).

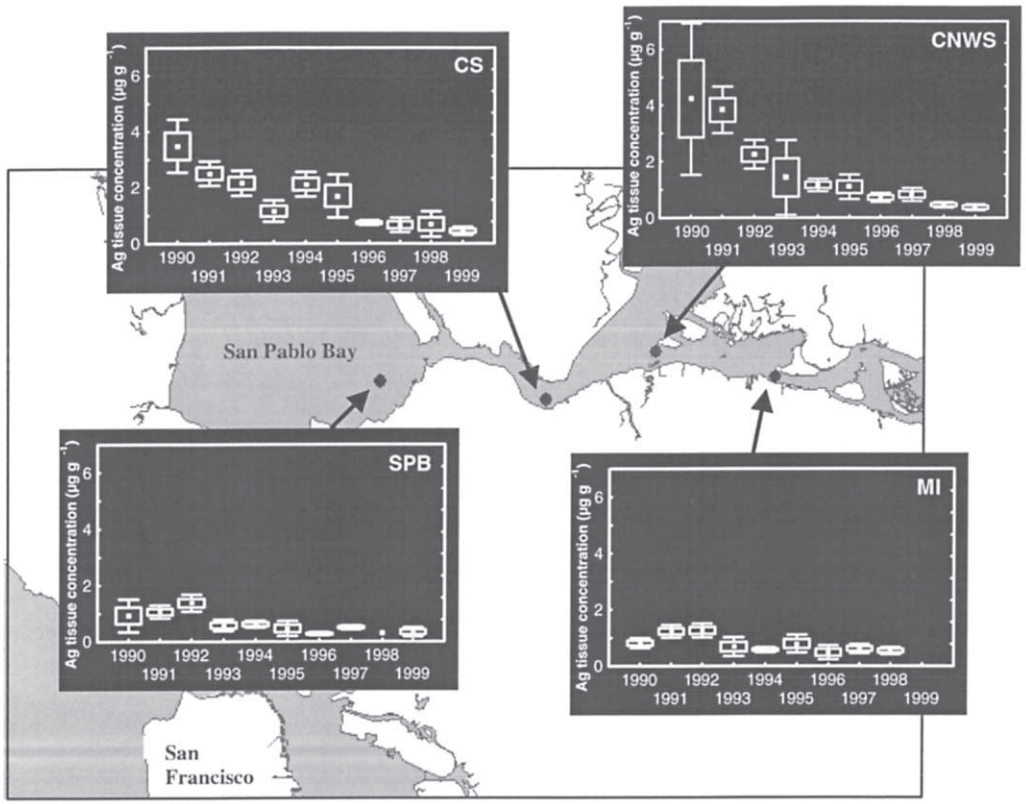

Figure 4. Annual mean silver concentrations (µg g^{-1} dry weight) in the tissues of *Potamocorbula amurensis* from 1990 to 1999 at each site. The boxes represent 1 standard error and the whiskers represent 1.96 standard error.

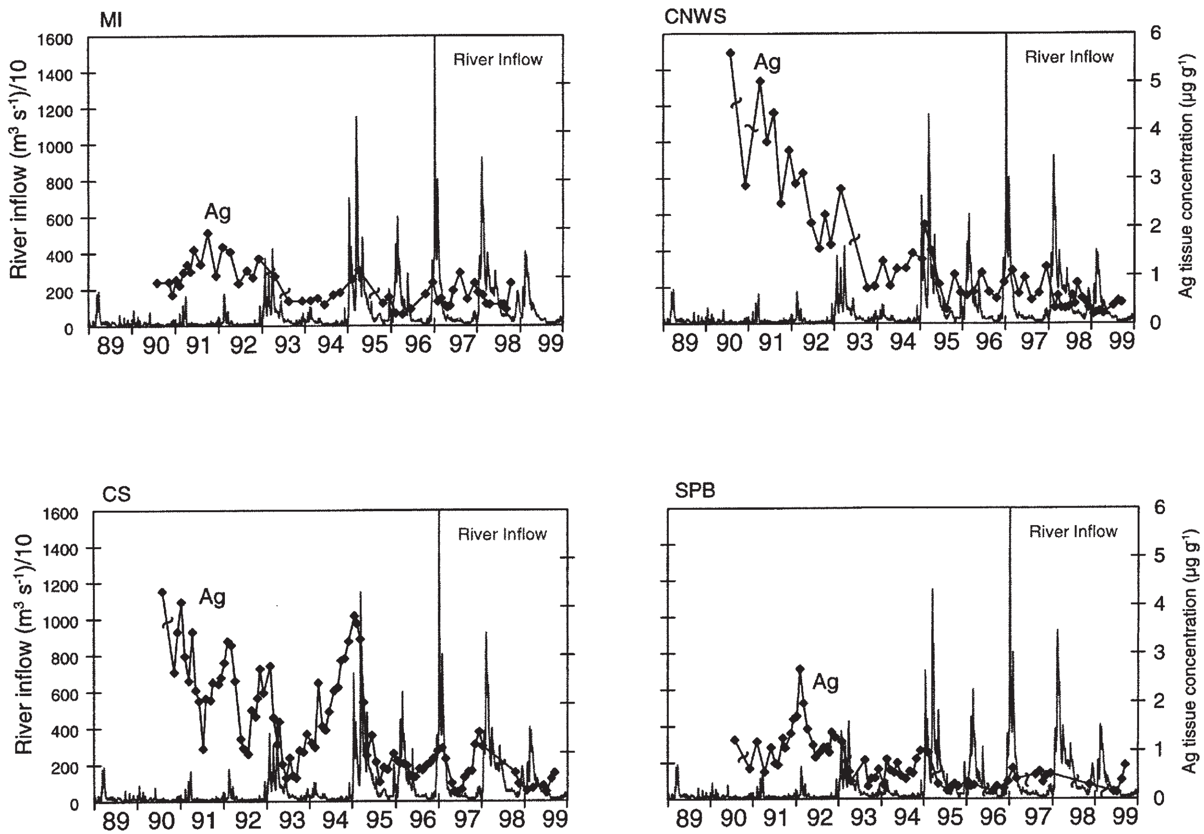

Figure 5. Monthly mean silver concentrations (µg g^{-1} dry weight, right axis) in the tissues of *Potamocorbula amurensis* at each site plotted with the river hydrograph (m^3 s^{-1}, left axis). The (~) indicates noncontinuous data.

related to the number of consecutive days that the flow remained below 1500 m^3 sec^{-1} (Figure 6). The coefficient of determination (r^2) indicated that 81% of the within site variability in maximum silver exposure (as indicated by tissue concentrations) was explained by the changes in flow conditions.

Reproduction Patterns in *Potamocorbula amurensis*

Monthly sampling was essential for identifying the reproductive status of the different populations because of the complex annual pattern of the maturation of gonadal tissues. Each population of clams from the four sites showed a different reproductive pattern over the length of the study (Figure 7). Clams at the MI and SPB sites showed similar patterns in their reproductive activity, where a majority of clams were reproductive (central tendency of reproduction was positive) 81% and 80% of the months sampled during the study, respectively. The months when clams were nonreproductive (central tendency of reproduction was negative) at these two sites occurred infrequently and were only one or two months in duration. The one exception to this was in 1992 when the length of time clams were primarily nonreproductive was 3 to 5 months (Figure 7) and the proportion of clams that were reproductive dropped to 36% (MI) and 60% (SPB) (Figure 8). In contrast to the MI and SPB sites, the populations at the CNWS and the CS sites were only reproductive 67% and 61% of the months sampled through 1997, respectively. The pattern of reproductive activity at these two mid-estuary sites was also different than at the MI and SPB sites. Clams were reproductive the first half of each year and non-reproductive the second half of each year (Figure 7). This pattern then shifted to a pattern where the proportion of months the clams were reproductive at CNWS and CS increased to values similar to those at the MI and SPB sites. This shift occurred after 1993 at the CNWS site and after 1995 at the CS site. Following these changes in reproductive activity, the proportion of reproductive clams at these two mid-estuary sites remained at 80 to 100% for the duration of the study (Figure 8).

Silver Tissue Concentrations and Reproduction in *Potamocorbula amurensis*

The timing of the increase in the reproductive activity at the two mid-estuary sites (CNWS in 1993 and CS in 1995) coincided with the timing of the final drop in Ag tissue concentrations in the clams at the same two sites (Figure 9). The decrease in reproductive activity at the SPB and MI sites in 1992 also followed a maximum in Ag accumulation. When Ag concentrations in the tissues were highest (annual mean > 1 μg g^{-1}), the clams were reproductively active 20 to 60% of the year. When Ag concentrations in the tissues of the clams were at an annual mean < 1 μg g^{-1}, the clams were reproductively active 70 to 100% of the year. A significant negative correlation between annual mean Ag tissue concentrations and reproductive activity was observed when all data were combined (aggregated annual means; Figure 10; $p< 0.05$). The data and linear regression analysis indicated that 59% of the variation in the reproductive activity of the clam is explained by changes in the Ag tissue concentrations.

DISCUSSION

The most likely source of silver to the northern portion of the San Francisco Bay estuary was from anthropogenic inputs. An internal source of Ag was indicated by

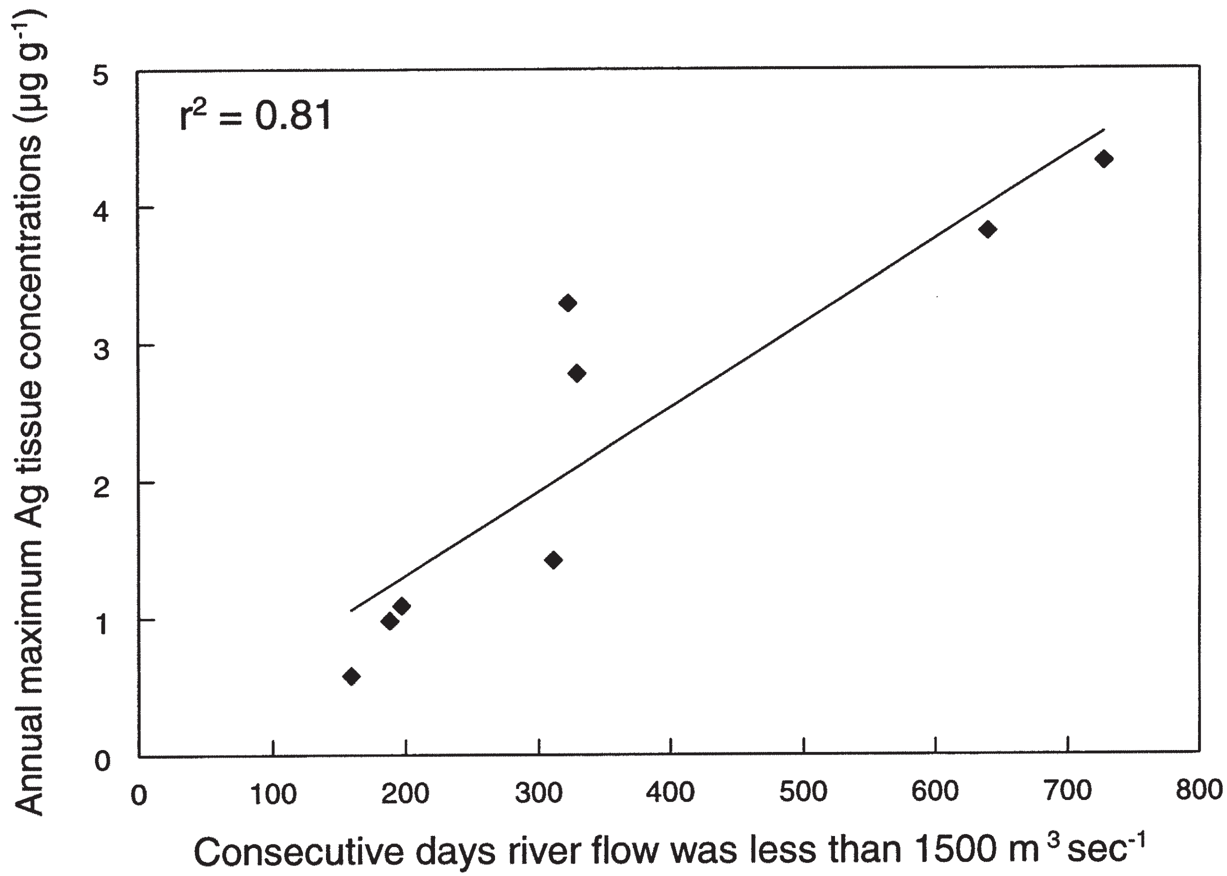

Figure 6. **Correlation between the number of consecutive days freshwater inflow from the Sacramento and San Joaquin rivers was low (below 1500 m^3 s^{-1}) and the annual maximum silver tissue concentrations (µg g^{-1} dry weight) in *Potamocorbula amurensis* during each of these periods of low flow.**

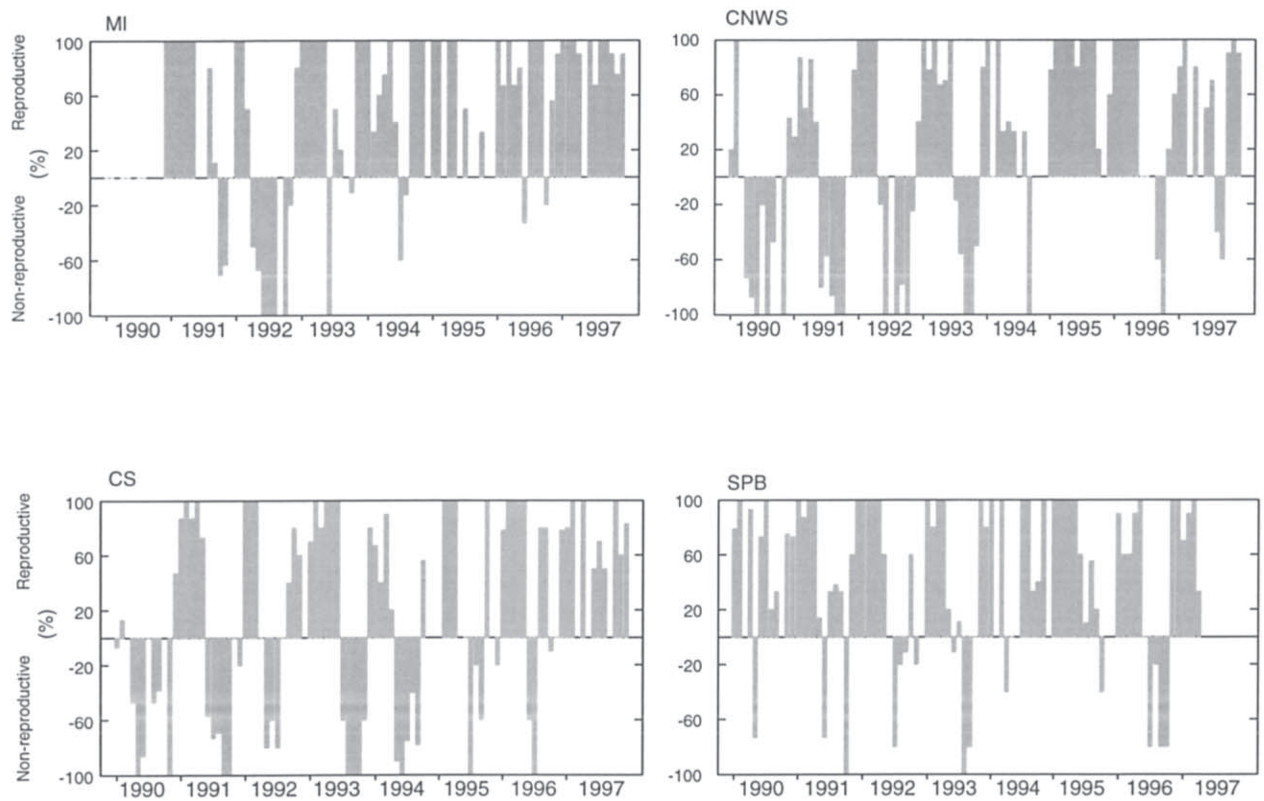

Figure 7. **Reproductive patterns of *Potamocorbula amurensis* at each site.** Bars represent the central tendency of the reproduction data: the proportion of clams that were reproductively active (% active + % ripe + % spawned) minus the proportion of clams that were nonreproductively active (% inactive + % spent) collected each month. Net reproductive populations are represented as positive bars and net nonreproductive populations are represented as negative bars.

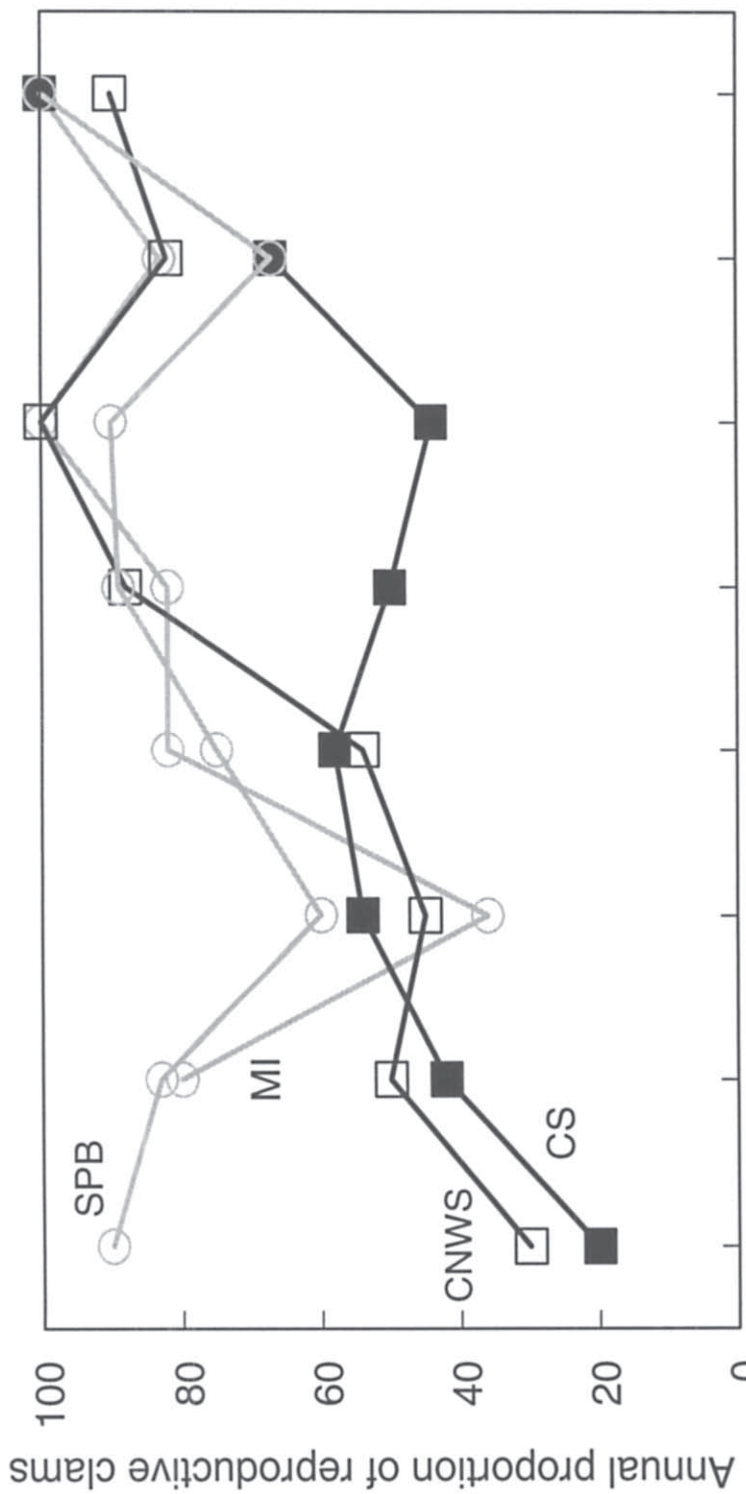

Figure 8. **The annual proportion of reproductive clams (*Potamocorbula amurensis*) at each site.**

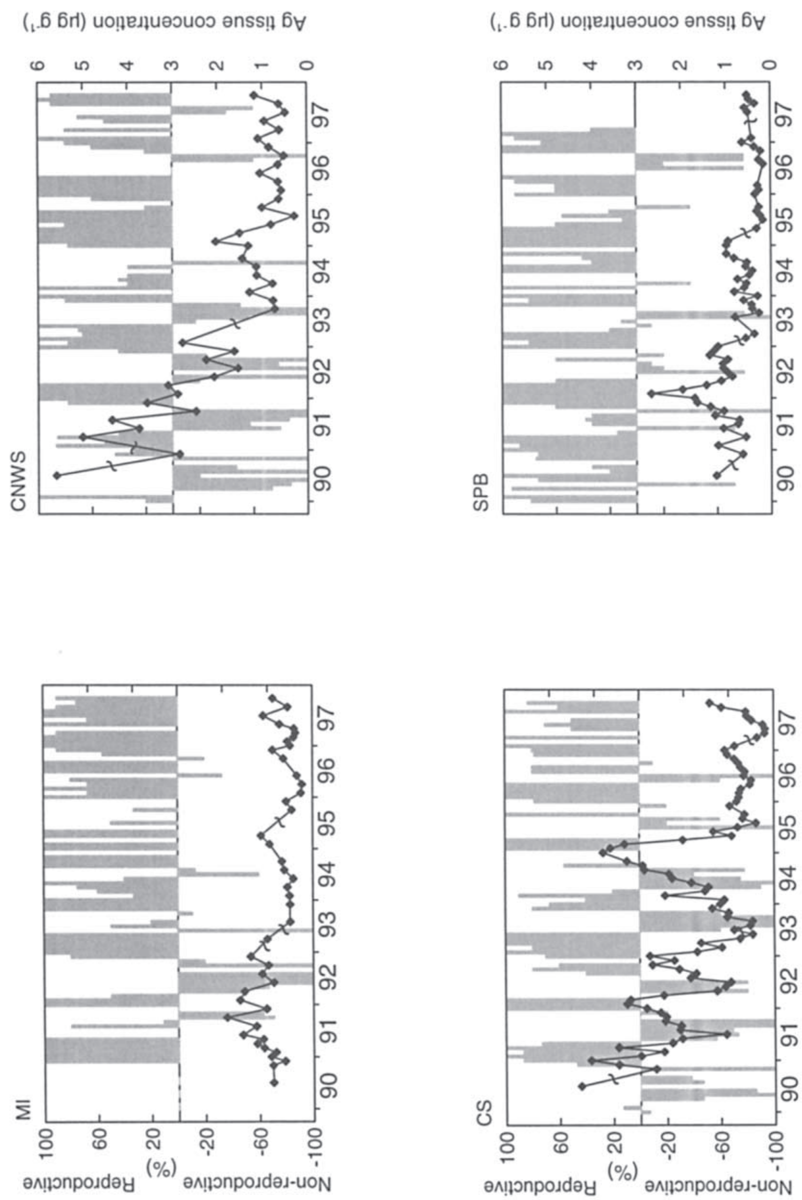

Figure 9. Monthly mean silver concentrations (μg g^{-1} dry weight, right axis) in the tissues of *Potamocorbula amurensis* at each site plotted with the central tendency of the reproductively active and non-reproductively active *P. amurensis* (left axis) at each site. The (~) indicates noncontinuous data.

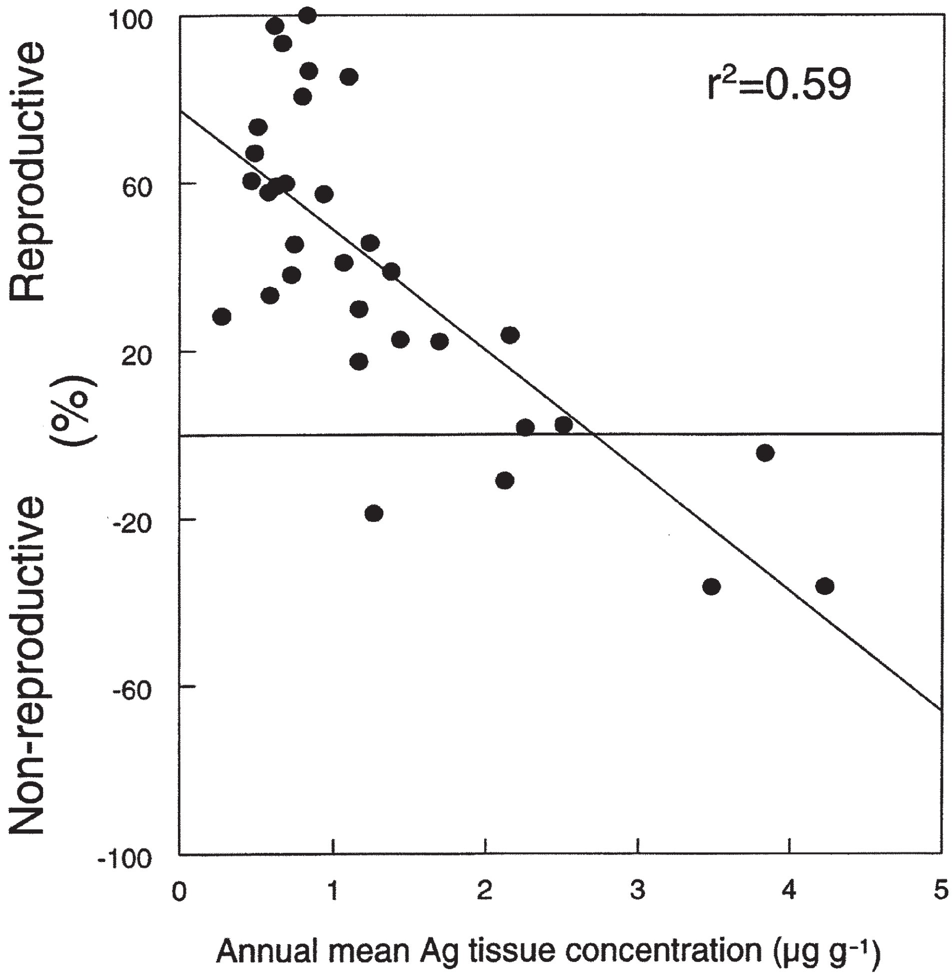

Figure 10. Correlation between the annual proportions (%) of reproductive clams (*Potamocorbula amurensis*) with the annual mean silver concentrations (µg g^{-1} dry weight) at the four sites. Y-axis represents the central tendency of the reproduction data: the proportion of clams that were reproductively active (% active + % ripe + % spawned) minus the proportion of clams that were non-reproductively active (% inactive + % spent) collected each month.

elevated tissue concentrations in *Potamocorbula amurensis* at the two sites located in the middle of the estuary (CS and CNWS). Natural occurrences of silver in the environment are rare, and were not a likely source. The average crustal concentration of silver is naturally low (0.1 ppm, Koide *et al.* 1986). Rivers have low dissolved concentrations, similar to oceans (Flegal and Sañudo-Wilhelmy 1993). Coring studies in the northern portion of the San Francisco Bay showed low background concentrations of Ag prior to the influence of human activities (Hornberger *et al.* 1999). Globally, potential sources of Ag include mining and processing of ore, photographic and electronic processes, cloud seeding and battery manufacturing (Purcell and Peters 1998). In fact, the presence of Ag contamination has been used as a common indicator of municipal and industrial discharges in past studies (Flegal *et al.* 1994; Smith and Flegal 1993; Sañudo-Wilhelmy and Flegal 1992; Ravizza and Bothner 1996). Many potential point sources of Ag occur along the shores of the northern portion of the bay. Monitoring reports indicate some enrichment of silver in dissolved suspended particulate and bed sediments in the area of Carquinez Strait during 1989 to 1993 (Flegal *et al.* 1994). Smith and Flegal (1993) also reported elevated sediment concentrations in 1991 in that area (1.16 $\mu g\ g^{-1}$ when compared with preindustrial concentrations of 0.02 $\mu g\ g^{-1}$ from sediment cores reported by Hornberger *et al.*, 1999). Silver in the effluent from a municipal sewage treatment plant on the Napa river (Figure 1) was reported at concentrations >100 $\mu g\ L^{-1}$. The Contra Costa County sewage treatment plant that discharges its effluent into the north San Francisco Bay between the CS and CNWS sites reported that dissolved Ag concentrations in their effluent were as high as 11 $\mu g\ L^{-1}$ prior to 1993 (personal communication).

Two factors could have contributed to the decrease in Ag concentrations in the clams over the length of the study. Decreased Ag in the discharge from point sources is a likely contributing factor. New restrictions were put into place for the reduction of Ag in wastewater from industrial sources in 1993. The Contra Costa County sewage treatment plant reported that silver in their effluent decreased from 11 $\mu g\ L^{-1}$ to 1 $\mu g\ L^{-1}$ in 1993 as a result of these new restrictions (personal communication). Differences in the hydrodynamic regime also occurred during this 10-year study period (Figure 5). Dry, low flow years were most common in the earlier years (1989 to 1992 and 1994); wet, high flow years were more common later (1993 and 1995 to 1999). Within the contaminated reach, Ag in the clams increased steadily during extended periods of low river inflow into the estuary, so the added influence of any local input could have been exacerbated by the low flows (presumably related to less flushing or longer residence times of water in the area).

Silver is an unusual element because both the dominant speciation reaction in seawater and the processes important in sorbing Ag on sediment particles enhance its availability (Luoma *et al.* 1995). Silver reacts with the chlorides in seawater and forms chlorocomplexes. These chlorocomplexes are bioavailable (Wang *et al.* 1996) and because they are in a dissolved state, enhance dispersal of Ag. Cycling occurs between Ag in the sediment and Ag in the overlying water by a combination of benthic flux of dissolved Ag into the water column (Smith and Flegal 1993), and scavenging of the Ag by particulates (and phytoplankton). If this re-cycling is most prominent during periods of lowest hydraulic flushing, *i.e.,* low freshwater input from the rivers, then it may continue even after Ag concentrations in wastewater discharges may have decreased. Although pockets of deposition may occur in Suisun Bay, it is a net erosional environment in the long term (Cappiella *et al.* 1999), which may explain the relatively rapid recovery at CS and CNWS. Bulk sediment Ag concentrations do not show large accumulations of Ag in this area, nor do they show clear temporal or spatial differences (Hornberger *et al.*, unpublished data). However, small changes in Ag concentrations in suspension could have the kind of impacts on bioaccumulation seen here (Luoma *et al.* 1995).

The following causal criteria described in Adams (2003) are used to describe the specific evidence that supports the relationship between the stressor, Ag, and the effect, change in reproductive activity, that was observed in this field case study.

Strength of Association

The pattern in reproductive activity of *Potamocorbula amurensis* was different among sites with different Ag tissue concentrations in the clams. The typical pattern of reproduction for bivalves in this estuary was demonstrated by the clams at the two end-estuary sites (MI and SPB). At these sites, individuals containing identifiable sperm or eggs were almost always present in the population, usually in a high proportion (average 80% of the months throughout the decade of study). That was not the case at CS and CNWS before 1995. A deviation in the typical pattern occurred at MI in 1992 and SPB in 1991, when there were several months of decreased reproductive activity (proportions dropped to <60%) (Figures 7 and 8). Interestingly, this coincided with slightly higher Ag tissue concentrations (although not to the level seen at the two most contaminated sites). However, when tissue concentrations decreased back to 1 $\mu g\ g^{-1}$ and remained so for the remainder of the study, annual mean reproductive activity returned to 80 to 100%.

The monthly sampling also identified different patterns of reproductive activity for *P. amurensis* at the two mid-estuary sites (CNWS and CS) than at the sites surrounding them. The lack of reproductively mature individuals in summer-fall coincided with the seasonal peak accumulation of Ag; so again, there was a strong association between the pattern differences and the occurrence of the stressor. The strength of association was made more convincing by the repetition of the association year-after-year over the 10 years, the tractable variability in Ag concentrations resulting from the large number of individuals analyzed and the monthly sampling (or bimonthly), which allowed a viable picture of the reproductive cycle and temporal variability in Ag exposure.

Consistency of Association

The association observed in this case study between Ag exposure as indicated by tissue concentration and changes in reproductive activity in a bivalve was also observed at a site in the southern portion of San Francisco Bay (Hornberger *et al.* 2000), in a different bivalve species, *Macoma balthica*. The south bay site was an intertidal mudflat compared with the deep-water channel of the North Bay study. Both sites were near outfalls of municipal sewage treatment plants that had reported high concentrations of silver in their effluents. The south bay study was also a long-term study but of even longer duration (1977 to 1999). Silver (along with Cu and Zn) was measured in *M. balthica* near monthly at one site located one kilometer south of a sewage treatment facility. Declines in tissue concentrations coincided with declines in effluent discharges from the plant. Monthly analyses of reproductive stage showed increased frequency of occurrence of eggs and sperm as Ag (and Cu) contamination declined. Other environmental variables did not show a unidirectional trend that correlated with the change in reproductive status. The remarkable consistency in biological response in these two episodes strongly points to Ag (perhaps in combination with Cu) as potential disrupters of reproduction in bivalves at concentrations well below those typically used in toxicity tests.

Specificity of Association

Many environmental factors can cause changes in reproductive activity in aquatic invertebrates. An organism can use changes in the environment as cues to synchronize reproduction with favorable conditions. A change in any factor an organism uses as a cue to begin reproduction could cause a change in the reproductive pattern. These factors can include salinity, temperature, or food conditions that are favorable for larval survival (Parchaso and Thompson 2002). However, these environmental factors were not influential in controlling the changes in reproductive activity in *P. amurensis* that were observed in this study. Changes in salinity in the northern reach of SFB would have affected the entire reach simultaneously. The shifts observed in reproductive activity occurred at two different times (1993 and 1995) and at two different locations (CNWS and CS). Parchaso and Thompson (2002) also reported that reproductively mature individuals of *P. amurensis* were found throughout the salinity range observed during the study, thus salinity is not a controlling factor of reproduction. Temperature can also be discounted as the cause for the changes in *P. amurensis* reproductive activity for the same reasons as mentioned above for salinity. Temperature changes would occur simultaneously throughout the northern reach of SFB, thus the shifts seen at the two different times (1993 and 1995) and locations (CS and CNWS) could not be explained by changes in temperature. Parchaso and Thompson (2002) reported that the onset of gametogenesis, maturation of gametes and spawning occurred in synchrony with periods of rainfall each year, regardless of the timing of the rainfall periods and thus regardless of the temperature within a given year.

Parchaso and Thompson (2002) concluded that *P. amurensis* might use the presence of various types of food as a cue to regulate its reproductive activity, and the differences between combined wet years and combined dry years (which were based on amount of river inflow) controlled the food type throughout the northern portion of the bay. However, when the reproductive activity during each month and year were examined individually, differences occurred in the reproductive patterns among sites that indicated factors other than type of food influenced the overall wet year versus dry year pattern. The difference in timing of the changes in reproductive activity between the two mid-estuary sites, CNWS in 1993 and CS in 1995, occurred during wet years. Similarly, the slight change in reproductive activity in 1991 and 1992 at the two end-estuary sites occurred during similar dry years. Changes in food type or availability would not explain the timing of such shifts. The only variable we know of at this time that could explain the timing of these shifts in reproductive pattern is the coincident changes in Ag tissue concentrations. Pooling the wet and dry years gave the primary driving force of food for the cue to reproductive activity; however the particular details of each year, regardless of wet or dry, coincide with changes in Ag concentrations in the tissues.

Contaminants other than Ag are also potential stressors in San Francisco Bay. But of the trace elements measured at the same time as Ag (Cd, Cr, Cu, Ni, Pb, V, and Zn), there was no indication, correlation, or similarity of pattern that would explain the changes observed in *P. amurensis* reproductive activity (Brown, unpublished data).

Organic contaminants can cause changes in reproductive activity. Tributyltin is a potent endocrine disrupting chemical and has potential for chronic effects on reproduction (Pereira *et al.* 1999). A study of butyltins (a subset of organotin compounds) in *P. amurensis* in the area of the CS site in 1995 found that *P. amurensis* accumulated mostly tributyltin (TBT) (123-166 ppb). There is, however, no information on the effect of TBT on *P. amurensis* reproduction. Reports on contamination in San Francisco Bay on studies of transplanted mussels, clams and oysters indicate that organotin contamination is widespread throughout San Francisco Bay, although at progressively diminishing overall levels (SFEI 1999). The sites at CS and CNWS are not near or in harbors so there is no reason to suspect that these were TBT hotspots, outside the level of contamination that would have affected all four sites. TBT contamination cannot be excluded as a confounding variable, but it seems unlikely based upon the geographic location of the sites. There are no studies available at this time on the environmental effects of organotin compounds on the biota in San Francisco Bay.

Research is being conducted on pesticide use and its fate in, and transport into San Francisco Bay (Kuivila and Foe 1995; Domagalski and Kuivila 1993; Bergamaschi *et al.* 2001; SFEI 1995). These organic compounds also could cause changes in reproductive activity, and further studies will be needed to evaluate their effects on the biota in the bay. Methods are currently being developed to measure a suite of organic compounds in *P. amurensis* (C. Orazio, personal communication). Again, the location of the affected sites make pesticides an unlikely source of the specific episode described here. Most pesticide inputs come from the rivers and are diluted along the estuarine gradient (Bergamaschi *et al.* 2001). The CS and CNWS sites were mid-gradient, and affected more than the site nearer the head of the estuary.

Time Order or Temporality

Time order is usually viewed in the sequence of adding a stressor and then observing a change. However, in the developed world the best opportunities for observing time order effects are probably during recovery from exposure to a stressor, as in this case study and Hornberger *et al.* (2000). Although such studies are perhaps not as rewarding as “detecting an effect”, these two studies show that recovery can provide clear evidence of how contaminants may be working elsewhere and the relationship between concentrations and effects, as well as providing positive evidence of the benefits of an action. The time order evidence in the present study was strong. A change in reproductive pattern was observed as Ag concentrations declined at CS and CNWS; and most convincingly animals returned to a reproductive status typical of less contaminated sites as the Ag contamination declined over the years. In fact, coincidence between decreased reproductive activity and increase in tissue concentrations of Ag in the clam occurred at all four sites, even though the increase in Ag contamination was slight at the least-contaminated sites.

Biological Gradient

The strongest evidence for establishing causality between Ag tissue concentrations and their effects on reproductive activity in *P. amurensis* with these field data is that the change in reproductive pattern occurred coincident with a change in Ag tissue concentrations both spatially and temporally (at different times among each of the sites). The changes in reproductive pattern did not co-vary among sites in a system where controls on reproductive activity appear to be related to system-wide rather than site-specific circumstances. At the CNWS site, Ag tissue concentrations were consistently high until 1993. Reproductive activity was low until the end of 1993. When Ag concentrations declined to approximately 1 $\mu g\ g^{-1}$ (similar to MI and SPB sites), reproductive activity increased to levels similar to levels in the clams at the MI and SPB sites. The CS site showed high Ag levels in *P. amurensis* until 1995, coincident with relatively low reproductive activity until 1995 when Ag concentrations declined to approximately 1 $\mu g\ g^{-1}$ and reproductive activity increased to levels similar to MI, SPB, and CNWS for the duration of the study. When all data (space and time) were plotted, a highly significant correlation was observed between tissue concentrations of Ag and reproductive status (Figure 10). Perhaps more important was the "factor ceiling distribution" of this data set (Luoma 1996). That distribution suggests that Ag caps the ability of the bivalves to produce viable sperm or eggs above a certain body concentration. This threshold body concentration was observed at 2.5 $\mu g\ g^{-1}$ where neither sperm nor eggs were found in the clams. Hook and Fisher (2001) observed Ag effects on reproduction in zooplankton at a tissue concentration of 0.7 $\mu g\ g^{-1}$. Much higher concentrations (approx. 65 $\mu g\ g^{-1}$) were required in *Macoma balthica* (Hornberger *et al.* 2000).

Experimental Evidence

Comparing experimental data to a particular field study can be difficult because dissolved concentrations rather than particulate forms are typically used in toxicity tests and because complex biological responses, like impairment of gonad development, can be difficult to elicit in the laboratory. Rarely do such tests assess the effect of contaminants accumulated through food. Form is critical to consider for Ag toxicity because of the particle-reactive nature and rapid uptake of Ag by phytoplankton, both of which may increase its availability (Wang *et al.* 1996). Hook and Fisher (2001) evaluated the sublethal effects of Ag on zooplankton from both dissolved forms and food. They also used concentrations similar to those observed in nature. The endpoints measured included mortality, growth rate, respiration rate, feeding rate, behavior, and reproductive success. The latter was measured as the number of eggs per individual multiplied by the hatching frequency per egg. There were no sensitivities observed in any of the measured endpoints when dissolved concentrations were below 10 $\mu g\ L^{-1}$. However, sublethal effects of Ag were evident after exposure following assimilation from food (phytoplankton exposed to dissolved concentrations as low as 0.1 $\mu g\ L^{-1}$). Of the endpoints measured in this study, the only one altered by exposure to Ag via food was reproductive success. Following dietary exposure, decreased egg production and viability occurred when tissue Ag concentrations increased.

The dissolved concentrations where Hook and Fisher (2001) saw reproductive effects (11 $\mu g\ L^{-1}$) were similar to experimental concentrations where *P. amurensis* larvae did not develop normally to straight-hinge stage (Brown and Nicolini, unpublished data). In the latter study, a preliminary laboratory experiment, designed from the study by Nicolini and Penry (2000), was conducted to explore the effect of Ag on larval development, from fertilization to the development of the straight-hinge larval state (48 h). It was found that the concentration needed to observe an effect (impairment on the development to the straight-hinge stage) was 10 μg L^{-1} (Brown and Nicolini, unpublished data). These dissolved concentrations were similar to Ag reported in the effluents discharged to the Bay, but are well below dissolved Ag concentrations observed in Bay waters themselves. The concentrations that caused effects in the Hook and Fisher (2001) study, via food (phytoplankton exposed to dissolved concentrations as low as 0.1μg L^{-1}), are more similar to concentrations feasible for a hotspot in a contaminated estuary, although no values that high have been measured in North Bay (maximum of 0.03 μg L^{-1}) (SFEI 1999). Little is known about effects from natural forms of Ag-contaminated food on reproduction in *P. amurensis*: from parent to offspring and on the development of gametes, larvae and young. The present work suggests study of such influences is warranted.

Biological Plausibility

Ag is one of the three most toxic elements (with Cu and Hg) to invertebrates and algae in marine and estuarine environments (Bryan 1984). Several experiments (Nelson *et al.* 1983; Eyster and Morse 1984; Calabrese *et al.* 1977; Martoja *et al.* 1988; Hook and Fisher 2001) support the biological plausibility of an effect of Ag on reproductive activity in aquatic organisms. Ag is shown to cause a premature release of gametes (Martoja *et al.* 1988), a reduction in larval releases and a reduction in the number of larvae (Nelson *et al.* 1983), a decrease in the number of eggs produced (Hook and Fisher 2001) and a decrease in the storage of glycogen that is necessary for reproduction (Martoja *et al.* 1988). Hook and Fisher (2001) suggested that Ag is an endocrine disruptor, at least in zooplankton. The mechanism for Ag toxicity on reproduction is believed to be the inhibition of vitellogenesis (the accumulation of yolk proteins) which is necessary for egg viability, and the focal point of ovarian development (Hook and Fisher 2001). Other effects on organisms are also documented. These include histopathologic effects such as yellowish to black particulate deposition in the basement membrane and connective tissue of various organs and tissues (Calabrese *et al.* 1984; Berthet *et al.* 1992), depressed growth (Calabrese *et al.* 1984), reduced condition index and reproductive impairments in mussels transplanted into south San Francisco Bay (Martin *et al.* 1984).

CONCLUSIONS

Silver is an element of importance in aquatic systems because it is highly toxic and is readily available through the combination of its reactivity with chlorides in seawater and the ease with which it is scavenged by particulates. The case study presented in this paper looked at the hydrology, tissue concentrations of silver and reproductive variability of the clam, *P. amurensis*, at four sites in a highly variable ecosystem. Such variability can create difficulty in understanding processes in a complex estuary such as San Francisco Bay. However, the high intensity, monthly sampling of a large number of individuals over a long period of time provided the opportunity to examine the factors that regulate availability of silver in an estuarine environment, and the effect silver may have on the reproduction of an aquatic organism.

The study showed that the source of Ag in the northern reach of the San Francisco Bay estuary was from a site-specific source in the middle region of the study area. Changes in silver accumulation in the tissues, driven by the hydrology of the estuary, coincided with changes in the reproductive activity of the clam. Reproductive activity increased when tissue concentrations of silver decreased. The weight-of-evidence presented in this paper strongly supports the cause and effect relationship between Ag and reproductive activity in *P. amurensis*. Most natural confounding factors (food, salinity, temperature) were eliminated as possible causative agents of the reproductive effects. A lack of information on organic contaminants prevents them from being discounted as possible stressors, but patterns of the effects in space and time were not consistent with what is known about these contaminants. Changes in flow conditions may be very important for the availability of silver to a system. If flow is limited, silver is more bioavailable (within a site, but not among sites), so hydrology may indirectly cause impairment to reproductive processes.

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