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Copper and Silver Accumulation in Transplanted and Resident Clams (*Macoma balthica*) in South San Francisco Bay

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ABSTRACT

Accumulation of Cu and Ag by soft tissues of the deposit-feeding clam Macoma balthica *was less than half in clams transplanted to a contaminated area than in clams native to that area. During a period of tissue growth, the transplants retained 50 % and 90 %, respectively, of the net Cu and Ag accumulated, but loss of metals from soft tissue by the resident population equalled net accumulation. Copper accumulation in the transplants did not occur during some periods when increases in the metal body burden of the resident population indicated that environmental exposures were high. The difference in metal accumulation of the two groups of clams may be the result of past environmental exposures. The results illustrate some limitations of using transplants as indicators of pollution events or of pollutant impact upon resident populations.*

INTRODUCTION

The use of bivalve molluscs as indicators of trace metal enrichment or biological stress in aquatic environments has gained widespread acceptance (Goldberg *et al.*, 1978; Bryan *et al.*, 1980; Phillips, 1980; Popham *et al.*, 1980; Jensen *et al.*, 1981; Thomson *et al.*, 1984). Most biomonitoring studies have employed bivalves native to the site of interest, but some studies have transplanted indicator organisms along a suspected pollution gradient (Bayne *et al.*, 1979; Simpson, 1979; Martin,

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et al., 1984). Transplanting organisms has some advantages. The same species may be placed at all experimental stations whether or not it is present naturally (Simpson, 1979). Transplants can also be taken from a single population, thus reducing genetic variability and improving the homogeneity of response among test environments. Questions remain, however, about how closely transplanted organisms follow the responses to contaminants of organisms native to an area. Bioaccumulation of trace metals is commonly observed when organisms are moved from an uncontaminated to a contaminated area (Bryan & Hummerstone, 1978; Simpson, 1979; Stephenson *et al.*, 1980; Bryan & Gibbs, 1983; Martin *et al.*, 1984). In some instances, metal concentrations in transplants appear to rapidly reach the same levels as those in natives (Bryan & Gibbs, 1983) but in other instances substantial differences in metal concentration between transplants and natives appear to remain for at least a number of months (Bryan & Hummerstone, 1978; Simpson, 1979).

In this study, the concentrations and contents of Cu and Ag are determined in native and transplanted bivalves having different exposure histories. Metal accumulation and loss by soft tissues is compared between the two groups during a 1·5-month period in the summer and a 6-month period during the winter and spring. The effects of transplantation on metal accumulation and tissue growth are also assessed.

METHODS AND MATERIALS

Study design

Deposit-feeding clams (*Macoma balthica*) were transplanted from a site in South San Francisco Bay that is less enriched with Cu and Ag (station 1) to a more enriched site (station 2, Fig. 1). Changes in metal concentrations in caged transplants and uncaged native clams (henceforth referred to as 'residents') were compared at station 2 over time intervals of 6 weeks to 6 months.

Concentrations of Cu and Ag in clam soft tissue fluctuate seasonally and inter-annually at both stations but exposure histories (i.e. level and duration of metal exposure) differ substantially between the two. Since 1975, Cu concentrations of *Macoma balthica* have regularly attained 300–600 $\mu g\ g^{-1}$ dry weight in winter at station 2 but have never exceeded 180 $\mu g\ g^{-1}$ in winter at station 1 (Luoma & Cain, 1979; Thomson *et al.*,

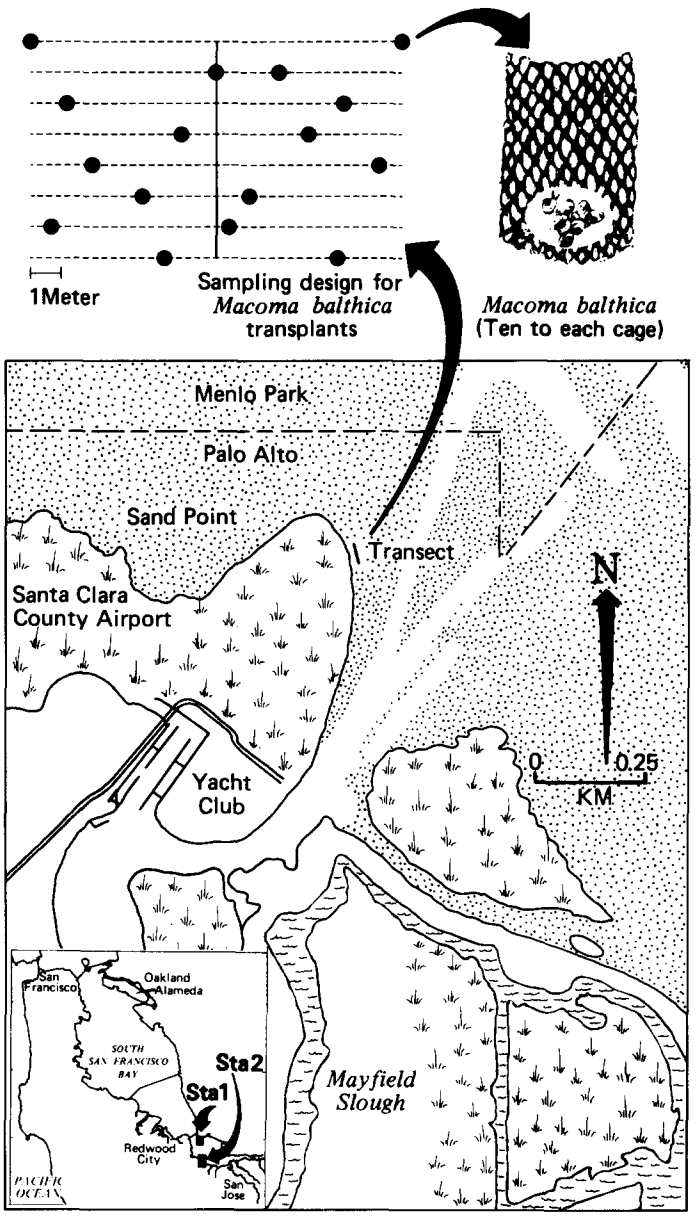

Fig. 1. Locations of stations 1 and 2 in South San Francisco Bay. Cages housing transplanted clams were arranged along a transect at station 2, as illustrated in the figure.

1984; S. N. Luoma *et al.*, unpublished data). Silver concentrations of nearly 200 $\mu g\ g^{-1}$ are common during the winter in *M. balthica* at station 2, but Ag does not exceed 15 $\mu g\ g^{-1}$ in *M. balthica* at station 1. *M. balthica* from station 2 show elevated tolerance to Cu and Ag compared with those from less contaminated sites elsewhere in San Francisco Bay (Luoma *et al.*, 1984; D. J. Cain *et al.*, unpublished data), reflecting their history of exposure to elevated metal concentrations.

Caging effects

Effects of caging on metal accumulation by *M. balthica* were examined in a separate experiment during the winter of 1981–82. *M. balthica* were collected at stations 1 and 2 and then re-buried in cages at the same stations. At monthly intervals, for three months, two cages from each site were retrieved, along with 20–30 clams not confined to cages. Metal concentrations of the two groups of clams, caged and uncaged, collected on the same day, were tested for significant differences ($p \leq 0{\cdot}05$) by the Mann–Whitney test (Sokal & Rohlf, 1969).

Transplantation from station 1 to station 2

Approximately 120 animals, ranging in shell length from 12 to 28 mm, were collected from station 1 on both 10 July and 28 November, 1978, and transferred to station 2. Animals collected on the 10th of July were used in a 6-week experiment during the summer. The experiment with the animals collected on the 28th of November was conducted throughout the winter–summer period. The summer and winter seasons differ in regard to metal bioavailability and seasonal growth of *M. balthica* in San Francisco Bay. The summer is typically a time of relatively low Cu and Ag bioavailability and stable or declining tissue weight, while the winter is a time of high Cu and Ag bioavailability in South San Francisco Bay (Luoma & Cain, 1979) and rapid tissue growth in *M. balthica* (Nichols & Thompson, 1982).

Transplanted animals were housed in cylindrical-shaped cages constructed of 1 mm plastic mesh, woven together with nylon twine. The cages were roughly 18 cm in height and 14 cm in diameter with closed bottoms and open tops (Fig. 1). Shell length of the transplants was measured and the animals were separated by length into 1 mm size classes.

An equal number of individuals from each size class was distributed among twelve cages. Each cage was filled, on site, with sediment from which all native *M. balthica* were removed by sieving. A numbered, plastic disk was placed near the top of each cage for identification, then the cages were randomly positioned adjacent to a transect parallel to the shoreline (Fig. 1). Pairs of cages were first placed 1 m apart along the transect, then moved 0–6 m away from the transect along a line perpendicular to it, one cage on either side of the transect. The distances the cages were positioned away from the transect were pre-determined by random selection. The cages were then buried so that their tops were just below the sediment surface. Between ten and twelve clams were placed within each cage and allowed to burrow down into the sediment.

Collection and analytical methods

On each sampling date, two cages, randomly selected by their identifying numbers, were collected from station 2. In addition, twenty uncaged station 2 residents were collected. The size range of the residents collected was the same as the transplants. All animals were depurated in sand-filtered seawater collected from Steinhart Aquarium, California Academy of Sciences, for 2 days at the existing field salinity. Shell lengths were recorded and the clams were pooled into 1 mm size classes; then the soft tissues were dissected from the shells. The soft tissues were weighed after drying at 70 °C for at least 24 h and then digested by concentrated nitric acid reflux (Bryan & Uysal, 1978; Luoma & Bryan, 1982). The digests were evaporated to dryness, reconstituted in 3N HCl and analyzed for Ag and Cu by flame atomic absorption spectrometry. Blanks were processed with the samples and used to correct sample concentrations. No more than one animal died in any of the cages during the experiments.

Tissue growth was followed in all experiments by plotting the weight of a 20 mm clam calculated from the allometric equation:

$$W = aL^b \tag{1}$$

where W is the dry weight of the soft tissues in milligrams, L is the shell length in millimetres and a and b are fitted parameters (Gallucci & Hylleberg, 1976). Seasonal change in tissue weight can affect trace metal concentrations and lead to spurious conclusions regarding metal accumulation and loss (Boyden, 1974; Simpson, 1979; Thompson, 1982;

Popham & D'Auria, 1983). Therefore, total metal content (micrograms of metal per individual) was calculated from:

$$C_{20} = (W_{20}) \cdot [M] \quad (2)$$

where W_{20} is the weight of a 20 mm clam calculated from eqn (1), $[M]$ the mean metal concentration of soft tissues ($\mu g\ g^{-1}$), and C_{20} is the metal content of a 20 mm clam.

Statistical methods

Changes in metal concentrations within the transplants and residents during the experiments were tested for significance ($p \leq 0{\cdot}05$) by ANOVA when the sample variances were homogeneous (analyzed by Bartlett's test) and by the Mann–Whitney test when they were not (Sokal & Rohlf, 1969).

RESULTS

Effects of caging

Figure 2 shows the effect of caging alone on the change in dry weight of clams between February and May at stations 1 and 2. The slight differences in the weight of the clams during the first month of the experiment may be an effect of disturbing the clams and sediment during the caging. The disparity in weight generally disappeared with the onset of growth.

Caging had no dramatic impact on metal concentrations or contents in tissues of *M. balthica*. Metal content (Fig. 3) of caged and uncaged clams was quite similar at each station except for Cu in April at station 2. Metal concentrations (Table 1) of caged and uncaged clams—including the Cu concentrations at station 2 in April—were never significantly different ($p > 0{\cdot}05$). The relatively high Cu concentration of uncaged clams at station 2 in April was influenced greatly by several older individuals which had extremely high tissue concentrations (similar to the 'super-accumulator' individuals described by Lobel *et al.*, 1982). In general, for samples collected on the same day, mean metal concentrations in uncaged clams fell within the differences in metal concentration observed between clams from different cages (Table 1).

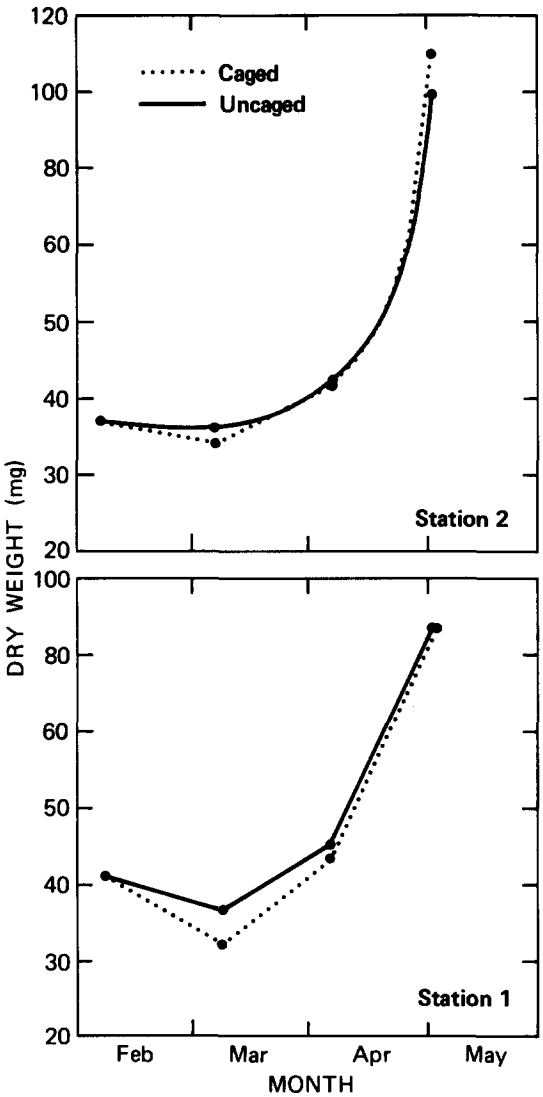

Fig. 2. Change in the dry weight of the soft tissues of caged versus uncaged *Macoma balthica* at stations 1 and 2. Dry weights were calculated for clams with a shell length of 20 mm.

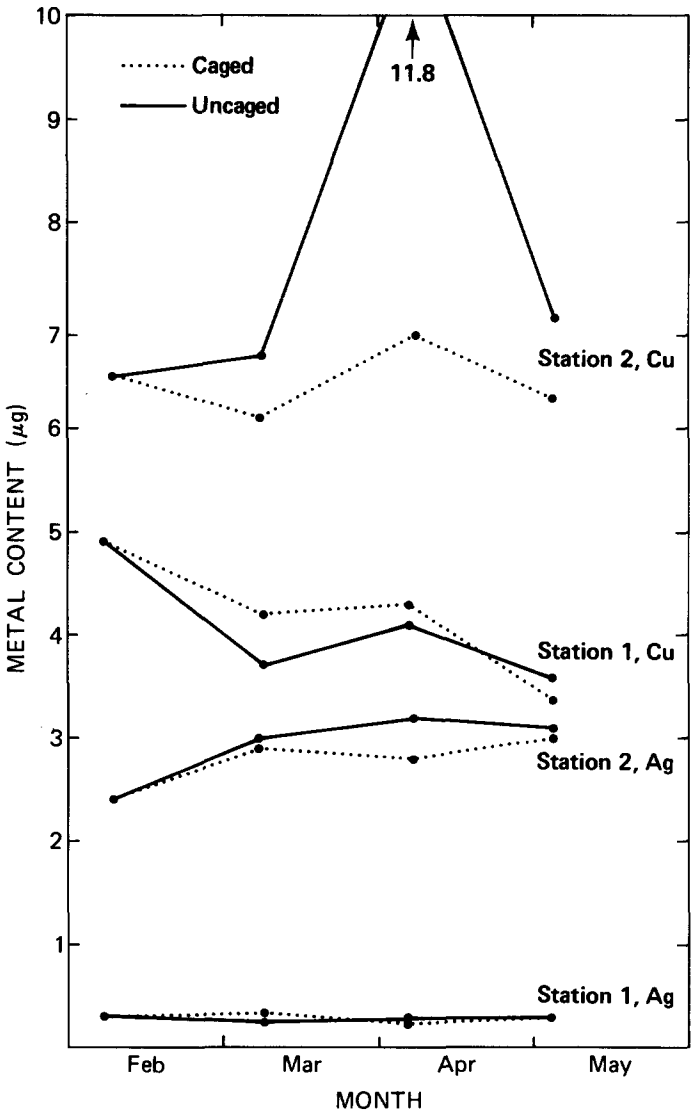

Fig. 3. Copper and silver contents (micrograms per individual) of the soft tissues of caged versus uncaged *Macoma balthica* at stations 1 and 2. Metal content calculated for 20 mm (shell length) clams.

TABLE 1

Mean Metal Concentrations ($\mu g\ g^{-1}$) $\pm$ One Standard Error of Caged and Uncaged *Macoma balthica* at Two Stations in South San Francisco Bay. Mean Concentrations for Individuals in Each Cage are Shown, as well as the Overall Mean for Both Cages. Number of Samples, *n*, is Indicated. Each Sample is a Pool of Individuals Having Similar ($\pm 1{\cdot}0$ mm) Shell Lengths

	Cage 1		*Cage 2*		*Cages 1 and 2*		*Uncaged*	
Month	*Cu*	*Ag*	*Cu*	*Ag*	*Cu*	*Ag*	*Cu*	*Ag*
February				*Station 1*			120 ± 22	7 ± 2 ($n = 7$)
March	188 ± 25	14 ± 2 ($n = 8$)	79 ± 6	6 ± 1 ($n = 9$)	130 ± 18	10 ± 1	102 ± 10	6 ± 1 ($n = 13$)
April	99 ± 11	6 ± 1 ($n = 9$)	96 ± 13	7 ± 1 ($n = 9$)	98 ± 8	6 ± 1	90 ± 10	6 ± 1 ($n = 13$)
May	56 ± 8	4 ± 1 ($n = 9$)	36 ± 6	3 ± 1 ($n = 10$)	45 ± 5	$4 \pm 0{\cdot}4$	49 ± 9	4 ± 1 ($n = 10$)
February				*Station 2*			204 ± 38	65 ± 16 ($n = 6$)
March	128 ± 12	47 ± 5 ($n = 5$)	310 ± 88	131 ± 47 ($n = 4$)	209 ± 48	84 ± 24	215 ± 25	84 ± 17 ($n = 6$)
April	170 ± 14	54 ± 5 ($n = 7$)	200 ± 26	77 ± 13 ($n = 8$)	190 ± 15	66 ± 8	278 ± 50	76 ± 15 ($n = 8$)
May	81 ± 20	33 ± 11 ($n = 7$)	92 ± 24	38 ± 10 ($n = 6$)	86 ± 11	35 ± 7	102 ± 33	39 ± 14 ($n = 9$)

Animals transferred from station 1 to station 2

The parameters a and b, derived from the allometric expression for tissue growth, varied seasonally in relation to the annual growth cycle, but the correlations of shell length and tissue weight were always significant ($p < 0{\cdot}05$).

Tissue weight calculated for 20 mm clams was approximately 70 mg for both the transplants and residents throughout the summer transplantation experiment (Fig. 4). The variation among the weekly samples in the summer probably reflects the accuracy of the method more than real

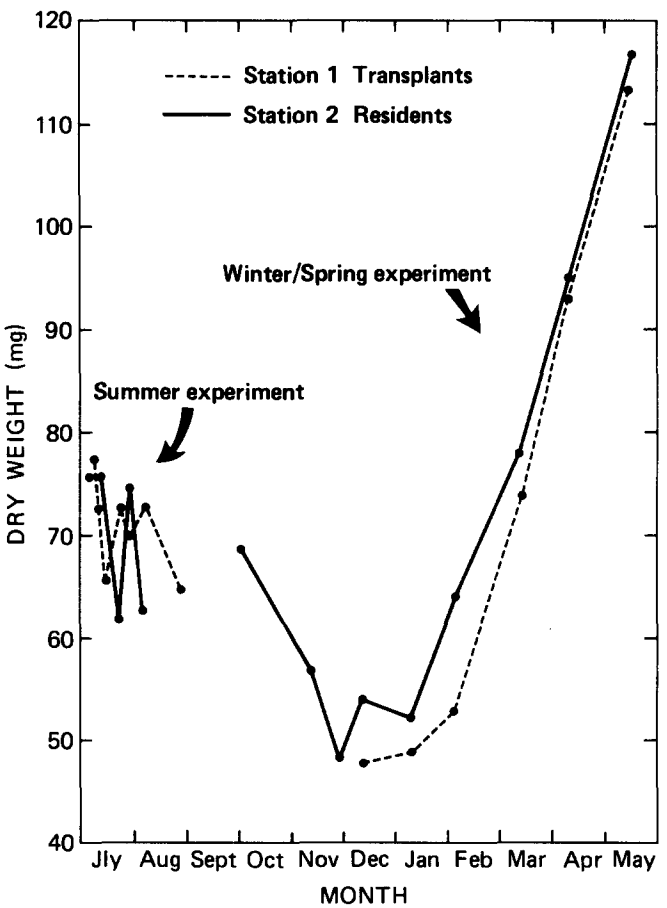

Fig. 4. Change in the dry weight of the soft tissues of 20 mm (shell length) resident and transplanted *Macoma balthica* at station 2.

changes in tissue weight. The datum for the last resident sample is not available because of errors in sample processing. In the second experiment tissue weight decreased in all animals throughout the autumn until the onset of growth. Other than the sample taken in February, tissue weights of transplants and resident clams were the same.

Copper concentrations of resident clams showed a slight increase over the course of the summer experiment (Fig. 5). A significant increase ($p < 0{\cdot}01$) occurred in November, shortly before station 1 animals were transplanted for the second experiment, but then no further significant increase in Cu concentration was observed. Copper concentrations of the transplants increased slightly during the summer. Concentrations displayed a trend similar to that of the resident population and were significantly higher at the end of the experiment. In the winter experiment, Cu concentrations in the transplants increased significantly between January and March but were stable during December and January when concentrations in residents were highest.

Ag concentrations increased significantly in both residents and transplants during the summer experiment (Fig. 6). During the winter, a significant increase in Ag was observed in the residents in November, preceding the experiment, and, in the transplants, between November and January. Although metal concentrations in the transplants were substantially less than those of the residents, concentrations of Cu and Ag decreased significantly in both groups of clams in the spring (Figs 5 and 6).

In general, trends in Cu and Ag content were similar to trends in concentration, except that the spring decline in concentration appeared to be exaggerated by tissue growth.

During the summer experiment, a slight increase in Cu and Ag content ($1{\cdot}0\,\mu$g Cu and $0{\cdot}6\,\mu$g Ag) occurred in both groups of clams. Resident clams were accumulating Cu and Ag in November before the introduction of transplants at station 2 and continued to accumulate these metals throughout the winter (Fig. 7). Net metal accumulation by the residents was approximately twice that of the transplants. All of the metal accumulated by the residents was lost during the spring. (The amount of Cu lost was almost ten times greater than that lost by the transplants.) The transplants retained over half the Cu and roughly 90% of the Ag accumulated during the winter.

Copper and Ag accumulated by the transplants was extrapolated over time to estimate how long it may take for a 20 mm transplant clam to

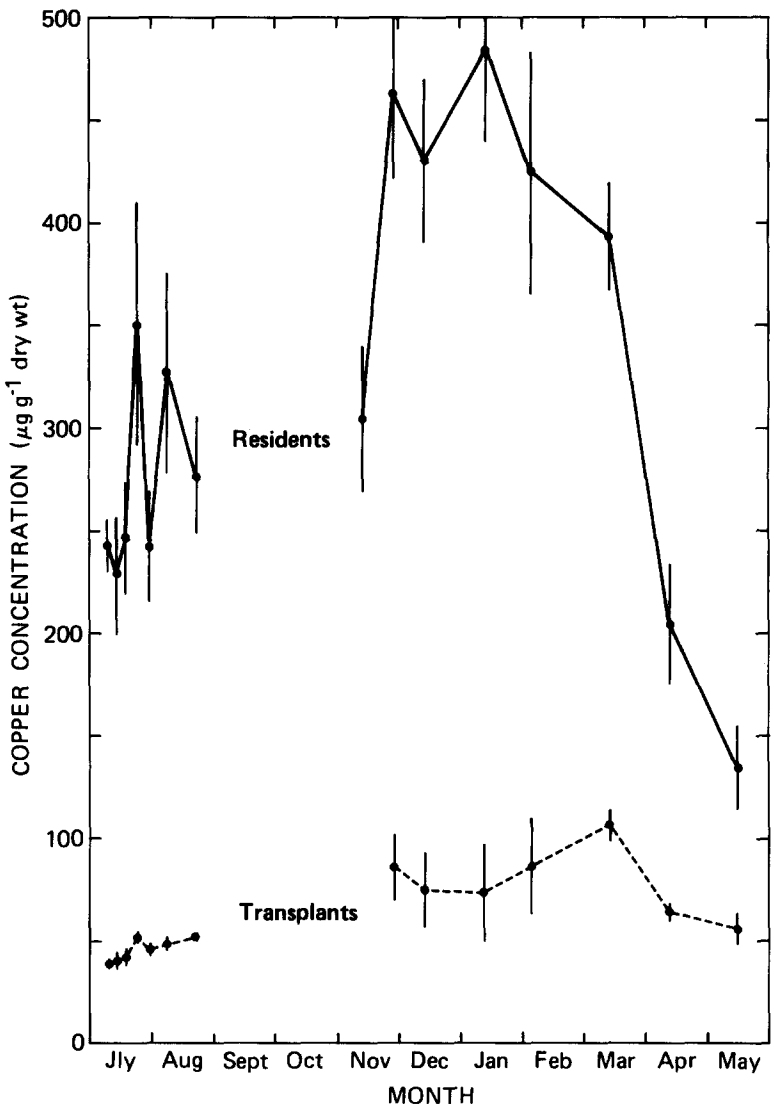

Fig. 5. Copper concentrations ($\mu g\, g^{-1}$) of resident and transplanted *Macoma balthica* at station 2. Bars indicate $\pm$ one standard error of the mean.

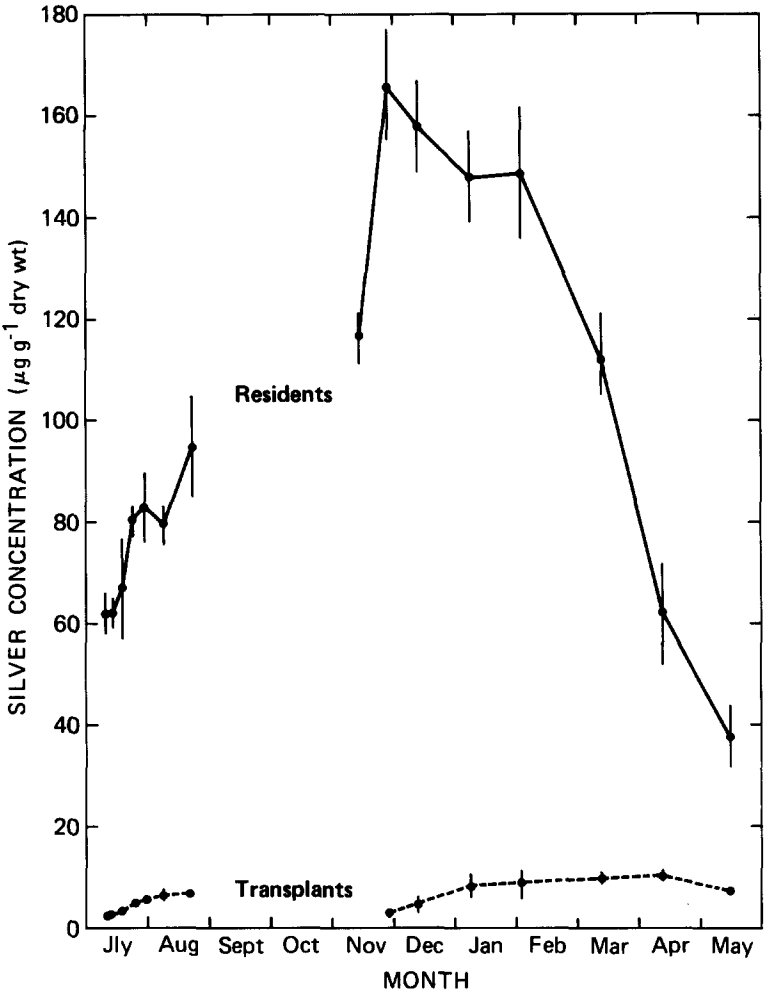

Fig. 6. Silver concentration ($\mu g\, g^{-1}$) of resident and transplanted *Macoma balthica* at station 2. Bars indicate ± one standard error of the mean.

reach a metal body burden roughly equal to a 20 mm resident clam (i.e. 20 μg Cu and 6 μg Ag: see Fig. 7). For these calculations, accumulation rates (micrograms per week) were calculated from the periods of significant metal accumulation observed during the summer and winter experiments (summer: 0·56 μg Ag and 1·0 μg Cu per 5 weeks; winter: 0·78 μg Ag per 19 weeks and 4·1 μg Cu per 9 weeks). The summer and winter seasonal rates for Ag and Cu were each extrapolated for 26

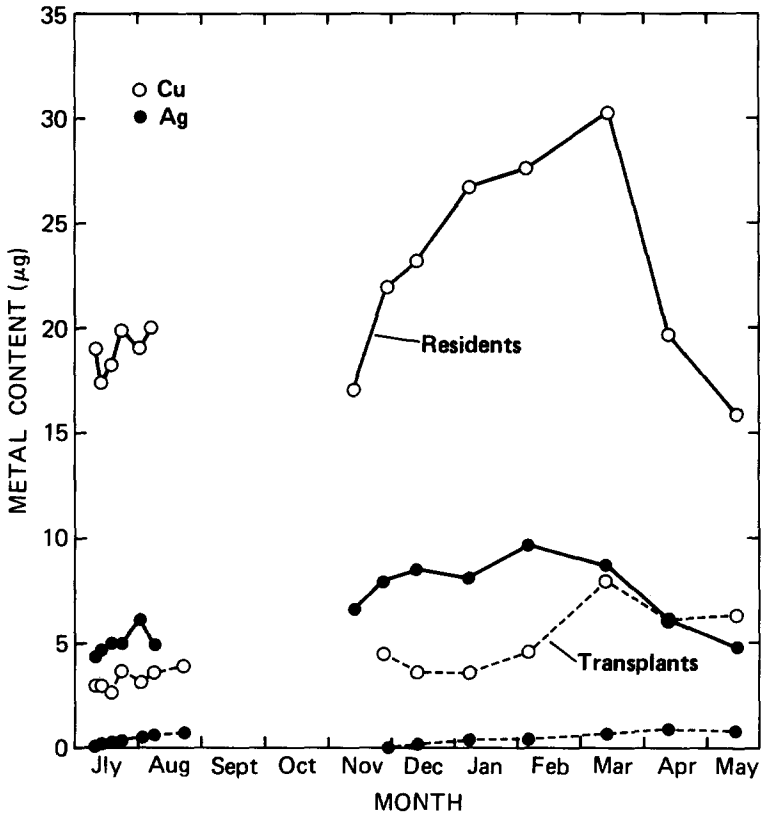

Fig. 7. Copper and silver contents (micrograms per individual) of resident and transplanted *Macoma balthica* at station 2. Metal content was calculated for a 20 mm (shell length) clam.

weeks assuming constant exposure at this rate (i.e. no periods of net metal loss). The calculations suggested that clams transplanted from station 1 would take a year before the Cu burden reached that of the residents and two years before the Ag burden was equal to that of the resident clams. The annual periods of metal loss or stabilization of metal accumulation could influence this result considerably.

DISCUSSION

Many biomonitoring studies implicitly assume that transplanted animals will show the same response to trace metals as might be expected of

residents. Effects of the transplantation itself, differences in growth patterns, different histories of metal exposure and differences in physiological or behavioral responses to metal exposure all could affect the validity of such an assumption.

Our results indicated that collecting and caging the deposit-feeding bivalve *M. balthica* had no detectable significant effect on metal bioaccumulation. Differences were observed in some samples between caged and uncaged animals from the same mudflat. The lack of consistent trends or statistical significance in such differences suggested that the transplantation process itself was not the cause of the differences we observed at station 2 in the metal responses of clams transplanted from station 1 and those of resident clams. Phases of the reproductive cycle appear to be synchronous in South Bay populations of *M. balthica* (J. Thompson, pers. comm.). The similarities in growth patterns observed during both the caging experiments and the transplant experiments suggested that seasonally changing biological processes (Nichols & Thompson, 1982) were in phase in the transplanted and resident clams and substantiated the lack of adverse effects from caging.

Growth affected Cu and Ag concentrations in both the transplants and the residents. Other authors (Phillips, 1976; Simpson, 1979; Popham & D'Auria, 1983) have suggested that total metal content is a more reliable indicator of metal contamination as it may eliminate growth biases. This method can add a bias, however, if the populations being compared have different size distributions (Popham & D'Auria, 1983). To eliminate both growth and size biases in comparing transplants and residents we employed a range of animal sizes and extrapolated metal contents to a standard size of animal (20 mm) from allometric correlations.

The history of an organism's metal exposure determines its body burden and may influence its physiological or behavioral response to metals (Ritz *et al.*, 1982; Worral & Widdows, 1983; Widdows *et al.*, 1984). When the transplant experiments were started, the Cu and Ag body burdens (content) of station 2 resident *M. balthica* were approximately six times and sixty times higher, respectively, than those of clams transplanted from station 1. Environmental levels of available Cu and Ag appeared to change during the study intervals at station 2. The quantity of metals accumulated during the second experiment was greater in the residents than in the transplants, but the loss of metal by the residents in the spring was also greater. The net effect was a reduction in the difference in body burden between the two to factors of about 6 for Ag and 2·5 for Cu.

The slower loss of Cu and Ag by the transplants could have been the result of their initially lower body burden. In the simplest sense, observed changes in metal burdens in animals are the result of net processes, reflecting the balance of gross fluxes. Metal content declines when gross influx falls below gross efflux. Metal clearance from an animal's soft tissues is proportional to the metal burden of those tissues and, in the absence of influx, can be described by a rate constant(s) representing the physiological process(es) governing efflux (Riggs, 1963; Beasley *et al.*, 1982; Schultz-Baldes & Cheng, 1979; D'Silva & Qasim, 1979; Roesijadi *et al.*, 1984; Fowler & Unlu, 1978). Any influx will reduce the net rate of clearance. This reduction will be greater, the lower the body burden of metal. Thus, a greater loss of Cu by residents than by transplants should be expected when environmental levels of Cu decline. The effect also should be more dramatic for Ag than Cu because the difference in prior exposure to Ag between residents and transplants (reflected in initial body burdens) was greater. Physiological differences between the populations (e.g. different efflux rate constants) could also cause differences in metal loss rates, but need not be invoked to explain the results observed in the spring.

The accumulation of Cu and Ag by the transplants relative to the residents was not consistent between the summer and the autumn. In the summer, similar quantities of Cu and Ag were accumulated by the two groups, but during the December–January period, when environmental exposure apparently increased, net accumulation by the transplants was much less than by the resident population. If uptake and loss rates were the same in the two populations (i.e. if the two metabolized metals similarly), the net quantity of metal accumulated should have been *greater* in the transplants due to their lower body burden.

Physiological differences, affecting either rates of uptake or loss, could account for the observed differences in Cu and Ag accumulation between the transplanted and resident *M. balthica*. Similar differences in rates of metal accumulation between mussel populations differing in past environmental exposure have been observed in both laboratory (Ritz *et al.*, 1982) and field transplant (Roesijadi *et al.*, 1984; Widdows *et al.*, 1984) studies. In the latter, differences in metal accumulation and loss between transplanted and native mussels were metal-specific and continued beyond the point when the transplants appeared to have acclimatized to their new environment. If metal exposures were similar, this implies that differences in the response between populations were

physiologically based and more intransient than some other compensatory adaptations.

Metal accumulation by the transplants may have been affected by behaviour, also. In laboratory experiments, the mussel *Mytilus edulis* (Abel, 1976) and the clams *Scrobicularia plana* (Trueman & Akberali, 1981) and *Macoma balthica* (D. J. Cain, unpublished data) close their shells when challenged by Cu. Eventually, the shells are reopened, but this behavioral response may effectively reduce the duration of exposure. Populations of *M. balthica* that are less tolerant to Cu (e.g. from station 1) close at lower level exposures than more tolerant populations (e.g. those from station 2; D. J. Cain, unpublished data), suggesting that the response is symptomatic of Cu toxicity. Metallothionein-like proteins (MLP), a class of low molecular weight, cysteine-rich proteins which bind strongly to trace metals, may play an important rôle in metal detoxification and tolerance in bivalves (Coombs, 1980; Roesijadi, 1980, 1982; George, 1983). The synthesis of MLP's can be induced by exposure to trace metals (Kohler & Riisgard, 1982; Viarengo *et al.*, 1981) and high concentrations of MLP's have been observed in species collected from metal-contaminated areas compared with control areas (Harrison & Lam, 1983; Viarengo *et al.*, 1982). A protein pool rich in Cu and Ag and having MLP properties has been found in *Macoma balthica* collected from station 2 (C. Johansson, D. J. Cain and S. N. Luoma, unpublished data). The differences in metal accumulation between the transplants and residents may be related to the maintenance of high levels of MLP's by the station 2 residents in response to chronic metal exposure. Assuming that the populations' responses were ecophenic (rather than genetically based), one could speculate that, since MLP's are inducible, metal accumulation rates of transplants during times of high metal exposure may eventually increase as the clams adapt (i.e. increase MLP synthesis) to the metal enriched conditions at station 2. Whatever the exact mechanism(s), transplanted and resident *M. balthica* appeared to be physiologically dissimilar in regard to their responses to trace metal exposure.

Liberal estimates (using maximum accumulation rates and assuming no period of reduced exposure) indicated that the time required for transplants to reach body burdens equal to those of residents would be at least 1 year for Cu and 2 years for Ag. These estimates could be greatly affected by environmental metal dynamics at station 2 but generally reflect differences in exposure history between the transplanted and

resident populations. In contaminated environments some metals may be progressively accumulated throughout the lifetimes of animals such as *M. balthica* (Strong & Luoma, 1981; Bryan & Hummerstone, 1978), and under such conditions metal concentrations in transplanted animals might never reach the levels of those in residents of similar age distribution (as observed by Simpson, 1979; Bryan & Hummerstone, 1978). Our estimate of 2 years to equalization of Ag levels is consistent with that conclusion, as the average lifespan of *M. balthica* in San Francisco Bay is thought to be only 2–4 years (Nichols & Thompson, 1982). However, in less contaminated areas (initial differences in metal body burdens between transplants and residents are smaller), concentrations in transplants might rapidly reach and continuously mimic, those in residents (as observed by Stephenson *et al.*, 1980; Bryan & Gibbs, 1983).

The utility of biological indicators as tools to monitor environmental pollution has been demonstrated in many studies. However, the results of this study and others indicate that the response to an environmental contaminant is population-specific and may be greatly influenced by the populations' histories of exposure. For this reason, transplanted animals used to compare levels of contamination at several locations must be selected from the same population (Ritz *et al.*, 1982; Widdows *et al.*, 1981). Even then, transplanted animals may not always provide a reliable indication of the timing of pollution events (e.g. note the differences we observed in Cu responses in November–March) or of the impact of pollution on local populations, especially when the differences in prior exposures to metals between transplants and residents are great.

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