



Fate, Bioavailability and Toxicity of Silver in Estuarine Environments

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The chemistry and bioavailability of Ag contribute to its high toxicity in marine and estuarine waters. Silver is unusual, in that both the dominant speciation reaction in seawater and the processes important in sorbing Ag in sediments favour enhanced bioavailability. Formation of a stable chloro complex favours dispersal of dissolved Ag, and the abundant chloro complex is available to biota. Sequestration by sediments also occurs, but with relatively slow kinetics. Amorphous aggregated coatings enhance Ag accumulation in sediments, as well as Ag uptake from sediments by deposit feeders. In estuaries, the bioaccumulation of Ag increases 56-fold with each unit of increased Ag concentration in sediments. Toxicity for sensitive marine species occurs at absolute concentrations as low as those observed for any non-alkylated metal, partly because bioaccumulation increases so steeply with contamination. The environmental window of tolerance to Ag in estuaries could be narrower than for many elements.

With Geoff Bryan's untimely death in September 1993, the scientific community lost one of its most insightful observers of how pollutants affect estuaries. Geoff had a unique ability to formulate important questions. His work showed an interdisciplinary appreciation of chemistry, ecology, natural history and biology. His curiosity made him both an excellent experimentalist and a keen observer of nature. He was unsurpassed at designing, conducting and interpreting field studies, where control is inherently difficult. His insights from those investigations have withstood the test of time and spurred the studies of others. Within the broader themes of Geoff's papers (especially his field studies), there were often sub-texts of insight that could too easily be lost. His observations about the distribution, fate and effects of silver are an example. Silver was included in most of his reviews and in several specific studies (Bryan & Hummerstone, 1977; Bryan & Uysal, 1978; Bryan, 1984, 1985; Bryan & Gibbs, 1985; Bryan & Langston, 1992), but was never itself a subject of any individual paper.

Silver is a contaminant of continuing scientific and management interest, but little has been published exclusively about the element. In this paper, the

chemistry, particle-reactions, bioavailability and toxicity of Ag in marine and estuarine waters are reviewed, building from Geoff Bryan's early observations. Illustrative data are presented from some heretofore unpublished experiments, from San Francisco Bay and from Bryan's many studies of estuaries from the UK. Each aspect of the environmental behaviour of Ag supports the view that it should be an element of concern as a contaminant in marine and estuarine ecosystems.

Materials and Methods

Previously unpublished experimental data are presented on two studies of Ag sorption to natural sediments and one study of Ag bioavailability to the clam *Macoma balthica*. Silver sorption was studied in experiments with surficial intertidal sediments collected from San Francisco Bay. The percentage of Ag removed from solution, or Ag accumulated in particles, was compared among untreated sediments and sediments from which components were removed by extraction. A control system with no particulate material was also maintained, but removal from solution was < 10%. The freshly collected sediments were first passed through a 100 µm sieve in 20‰ seawater at 12°C (also the experimental conditions). In the first experiment, aliquots of sediment (2 g dry wt) were split into centrifuge tubes. Four tubes of sediment were employed for each treatment. Treatments included 1. repeated extraction of sediment (three times) with 0.1 M NaOH to strip natural organic materials followed by repeated washing in seawater; 2. extraction with 0.1 M NaOH followed by extraction with acid ammonium oxalate (repeated three times), to strip organic material and amorphous iron/manganese oxides; 3. sterilization in an autoclave; and 4. untreated. Sediments in each tube were transferred to replicate 250 ml flasks containing 100 ml of seawater (50:1, solute/solid ratio) with 800 nm silver (80 µg l⁻¹) in solution. Dissolved Ag was analysed by graphite furnace atomic absorption spectrophotometry, to determine Ag removal from solution through time.

A second series of experiments was conducted in which sediments were repeatedly extracted with 0.25 M hydroxylamine hydrochloride in 25% acetic acid

(HH+HAc). The surface area of the sediments was estimated before and after treatment by BET analysis of N adsorption on freeze-dried sediments. Acridine orange staining was used to semi-quantitatively estimate the removal and regrowth of bacteria. Concentrations of Fe and Mn extractable by weak (0.5 M) HCl were analysed to determine removal or mobilization of the most amorphous forms of the oxides. Treated and untreated sediments (1 g dry wt) were mixed into 1000 ml of seawater (1000:1, solute/solid ratio) containing 250 nM Ag ($25 \mu\text{g l}^{-1}$) and allowed to settle in a thin layer on the bottom of the beaker. Preliminary experiments showed that anaerobic conditions did not develop in sediments under such conditions over as long as 2 weeks. Particles were analysed after either a 2 h extraction in 0.5 M HCl, or after near-total decomposition by nitric acid reflux (Luoma & Bryan, 1978). All experiments were conducted in the dark.

Bioaccumulation of silver in the bivalve *M. balthica* was studied by spiking sieved sediments for 24 h. Sediments were obtained from the oxidized surface of a mudflat, as above, and spiked as discussed above. Uptake was compared between untreated sediments and the same sediments treated (as above) with NaOH and acid ammonium oxalate, to remove iron oxides and organic materials. Clams were exposed to sediments spiked to Ag concentrations ranging from 0.2 to $4.8 \mu\text{g g}^{-1}$ dry wt (determined by extraction with 0.5 M HCl). Pre-treated sediments sorbed $1.5 \mu\text{g g}^{-1}$ HCl-extractable Ag and $17 \mu\text{g l}^{-1}$ Ag was in solution after the exposure period. The untreated sediments sorbed $1.9 \mu\text{g g}^{-1}$ Ag and $< 1 \mu\text{g l}^{-1}$ was in dissolved form. Fifteen clams were sampled at each interval from each treatment, and soft tissues were separated from the shell. Fifteen clams were also held on a mesh above the sediments and analysed at the end of the experiment. The animals were dried, weighed, dissolved by concentrated nitric acid reflux (Luoma & Bryan, 1978), reconstituted in 0.5 M HCl (a critical step in Ag analysis), and analysed by AAS. These experiments were also conducted in the dark.

The chemistry of silver in estuarine and marine environments

Silver is a geologically rare element. Nevertheless, it is a contaminant in some types of mining and smelting wastes, and is found associated with many sewage discharges, especially in marine environments (Martin *et al.*, 1988; Bryan & Langston, 1992; Sanudo-Willhelmy & Flegal, 1992). In freshwater, thermodynamic models indicate that the monovalent silver ion is a dominant dissolved species (Wingert-Runge & Andren, 1993). However, the concentrations of Ag in solution in freshwaters are usually low. Silver binds strongly to surfaces in low ionic strength waters and can precipitate stoichiometrically as $\text{AgCl}_{(s)}$ if traces of chloride are present. Thus, Ag is rarely cited as a pollutant of concern in freshwater.

The salinity of brackish and marine waters greatly affects the chemical behaviour of Ag. Thermodynamic models show that strong chloro complexes (AgCl^0 , AgCl_2^- , AgCl_3^{2-} , AgCl_4^{3-}) dominate speciation (Cowan *et*

al., 1985). Thus, instead of precipitating in estuaries and marine environments, or remaining in the form in which it is introduced, chloro-complexation favours the retention of at least some Ag in dissolved form.

Dissolved Ag in marine waters is an effective indicator of anthropogenic inputs (Sanudo-Willhelmy & Flegal, 1992), partly because its chemistry favours a relatively stable dissolved form that can be dispersed. Natural Ag sources are virtually absent from coastal and estuarine waters (Sanudo-Willhelmy & Flegal, 1992). Nor is Ag recycled as readily from decaying cells as Cd, for example, although both metals form chloro-complexes (Lee & Fisher, 1992). However, dissolved Ag is widely dispersed around anthropogenic input sources. Anthropogenic Ag was identified in a 250 km gradient along the coast of southern California and northern Mexico (Sanudo-Willhelmy & Flegal, 1992); and regional-scale enrichment of dissolved Ag has been identified in South San Francisco Bay (Smith & Flegal, 1993), San Diego Bay (Sanudo-Willhelmy & Flegal, 1992), and the Severn estuary in the south-west of the UK (Bryan, 1985).

The association of Ag with particulates in marine waters

The accumulation of Ag by complex sediments. The chemistry of Ag in solution should affect its association with particulate materials. Chloro complexation could reduce sorption in marine waters, compared to freshwaters. Working with well-defined surfaces, Davis (1977) demonstrated that $< 5\%$ of Ag was adsorbed by iron oxides (goethite) in 15 h at pH 8 and 9.4×10^{-2} M chloride (equivalent to $\sim 5\%$ salinity). In contrast, 80–90% of Ag was adsorbed at pH 8 in the presence of traces of chloride (concentrations of 10^{-3} M) in 15 h. In the latter 'freshwaters', most adsorption occurred within 5 min.

Chloro complexation does not prevent the association of Ag with natural particles, unlike pure iron oxides. In dense suspensions of oxidized estuarine sediments, Ag is efficiently stripped from solution. Two grams of oxidized estuarine sediment removed $> 99\%$ of the Ag from 100 ml of a solution of $80 \mu\text{g l}^{-1}$ Ag within 24 h at 20‰ salinity (Fig. 1). Reactive surfaces on natural oxidized particles include complex aggregates of iron oxides, manganese oxides and various types of organic materials in estuarine sediments (Jenne, 1977). The rate of Ag removal from solution in the dense suspensions was reduced when such coatings were removed (Fig. 1). The rate of Ag sorption was slower than in untreated sediments when the most labile organic coatings were removed by 0.1 M NaOH extraction. If both organic material and amorphous iron oxides were removed from the sediment, sorption was yet slower. The greater the variety of components removed, the greater the effect. Sterilization of the sediment had the least effect on the Ag removal rate.

If the sediment-to-solution ratio was increased to 1000:1, sediments stripped of their coatings did not accumulate as much Ag as untreated sediments, even over a 2 week period. Pre-treatment with hydroxylamine in acetic acid efficiently removed bacteria, and stripped 90% of amorphous Fe and Mn from the

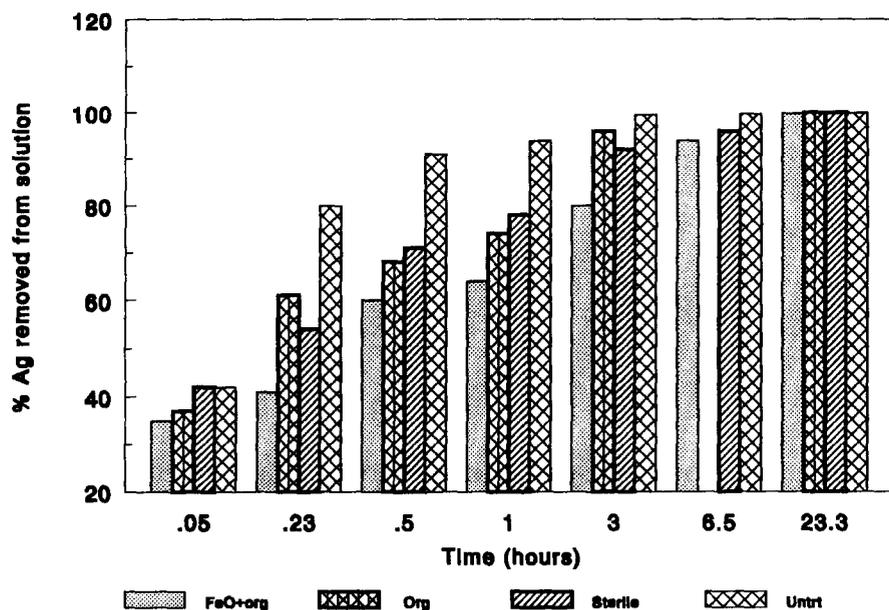


Fig. 1 Removal of Ag from solution by oxidized estuarine sediments. Untreated sediments (untrt) are compared to sediments from which organic materials were stripped (org); iron oxides and organic materials were stripped (FeO+org); and with sediments that were sterilized before being exposed to Ag.

sediments. It also resulted in a 25% increase in the sediment surface area (presumably because clay surfaces were exposed; see Fig. 2). Less Ag accumulated on these stripped sediments than on untreated sediments, despite the presence of a greater surface area.

The sequestration of Ag increased gradually over 2 weeks on both treated and untreated sediments. Partial re-growth of the bacterial coatings occurred during the 2 week experiment, but concentrations of Fe and Mn did not change. The lack of change in redox-sensitive Fe and Mn suggested anaerobic conditions did not develop. There may have been some effect from the bacterial regrowth and coating of sediments on pre-treated sediments, where Ag concentrations increased more rapidly than on untreated sediments. However, a kinetically slow component of sorption may also be characteristic of Ag reactions with particle surfaces (Davis, 1977). Throughout the experiment, 55–85% of the particle-bound Ag was extractable by HCl. This proportion was highest initially, and declined with time in both treatments.

The experiments with natural sediments were quite simplistic, but showed that Ag in seawater reacts with natural particles and most readily with the removable aggregates that coat the surfaces of such particles in nature. Removal of individual sediment components did not reduce the Ag-sediment association as much as removal of the suite of components. Greater than half of the Ag was itself removable by weak acid immediately after sorption, under these conditions.

Silver in sediments from nature. Silver also reacts with sediments in nature. This is evidenced by a net accumulation of Ag in the sediments of estuaries affected by human activities. Figure 3 shows Ag concentrations in 2 m sediment cores near the mouth of urbanized San Francisco Bay, compared to concentrations in a core

from Tomales Bay (data from M. Hornberger *et al.*, US Geological Survey, in prep.). The watershed of Tomales Bay is small, and is developed mostly for agriculture. Concentrations of Ag in San Francisco Bay in horizons deeper than ^{137}Cs penetration (before 1955) show concentrations similar to those from Tomales Bay. Both indicate that 'background' concentrations of Ag in sediments of the San Francisco Bay region are about $0.1 \mu\text{g g}^{-1}$ dry wt. Silver has accumulated to more than 15-fold that concentration in the surface layers of sediments at the mouth of San Francisco Bay, coincident with human activities.

Silver can reach concentrations of $0.5 \mu\text{g g}^{-1}$ and in some cases $> 1.0 \mu\text{g g}^{-1}$ dry wt in cores taken in anoxic sediments from undisturbed environments (Koide *et al.*, 1986). The highest natural concentrations occur in sulphidic hydrothermal deposits. Concentrations are closer to $0.1 \mu\text{g g}^{-1}$ dry wt in crustal rocks and deposits formed under oxic conditions. The background concentrations of $0.1 \mu\text{g g}^{-1}$ in the San Francisco Bay and Tomales cores suggest these sediments were (and are) oxidized at the surface, as the Ag was deposited. This is consistent with contemporary conditions.

Surveys of a wide variety of English estuaries (Bryan, 1984; Bryan *et al.*, 1985) also showed Ag concentrations in surficial, oxidized sediments of about $0.1 \mu\text{g g}^{-1}$ dry wt in the least-contaminated environments. Concentrations of Ag in sediments associated with widespread anthropogenic disturbance range up to $5 \mu\text{g g}^{-1}$ dry wt (Fig. 4). A few environments occur where Ag concentrations exceed $100 \mu\text{g g}^{-1}$ dry wt in sediment, but these are very exceptional (Bryan, 1984). Absolute concentrations of Ag in contaminated sediments are comparable only to Cd among the more common pollutants, but are low compared to more abundant elements. Sedimentary Ag enrichment of 50-fold ($5 \mu\text{g g}^{-1}$ vs a background of $0.1 \mu\text{g g}^{-1}$ dry wt) in polluted

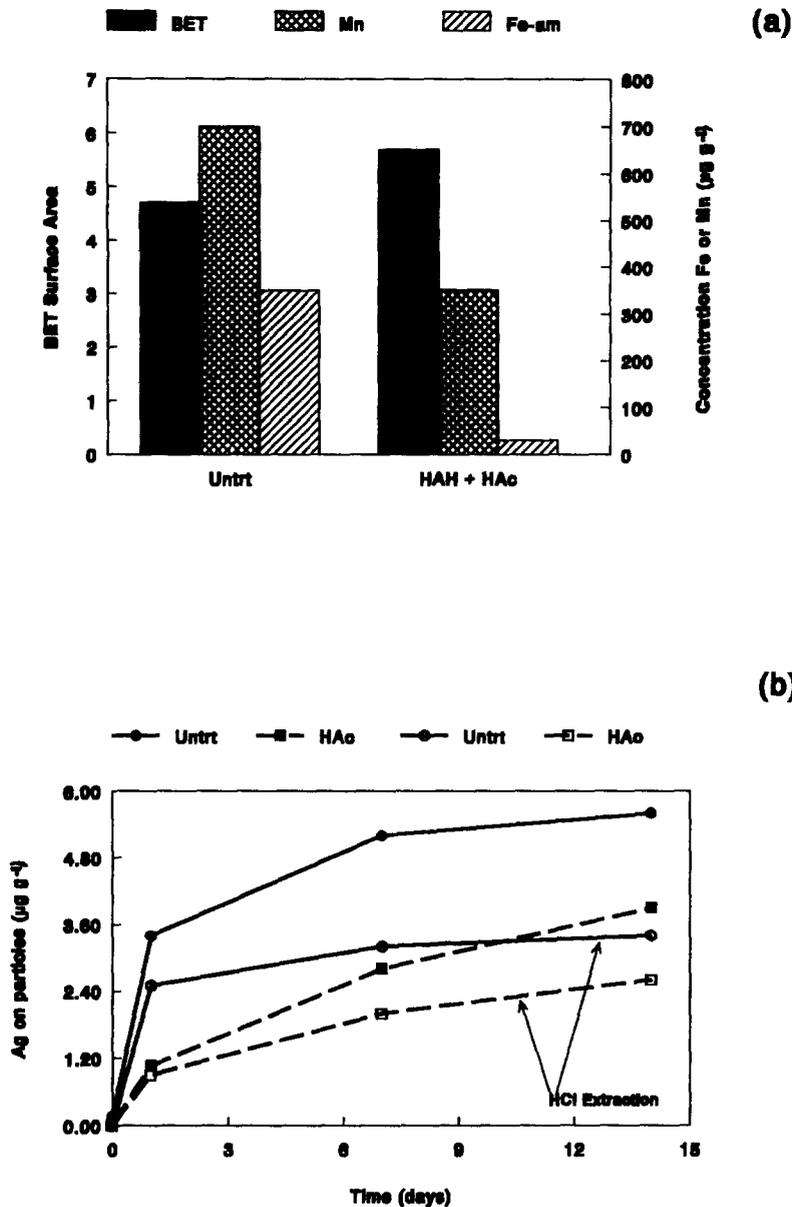


Fig. 2 (a) Characteristics of untreated oxidized estuarine sediments and sediments treated with hydroxylamine hydrochloride in 25% acetic acid. BET, surface area; Mn, Mn extracted by 0.5 M HCl; Fe-am, Fe extracted by 0.5 M HCl. (b) Accumulation of Ag on untreated oxidized estuarine sediments and on sediments treated with hydroxylamine hydrochloride in 25% acetic acid (HAc). Ag concentrations on particles were determined after near total decomposition with concentrated nitric acid reflux (closed symbols) and after 0.5 M HCl extraction (open symbols).

estuaries is comparable to other types of trace element contamination.

Luoma & Bryan (1981) used extractions and statistical relationships to assess the forms of Ag in oxidized surface sediments from 17 English estuaries. Both approaches were limited by their operational nature. Nevertheless, both suggested an association of Ag with the types of sediment coatings that enhanced sorption in the laboratory experiments. A high proportion of Ag (compared with many metals) was extracted with humic substance-type organic materials (10–20% of Ag was soluble in 1 M ammonia). Some 25–40% of the Ag was removed by extraction with either 0.5 or 1 M HCl. It is notable that less Ag was HCl-soluble in nature than in sediments that recently sorbed the element (compare to the adsorption experiments

shown in Fig. 2b). The concentrations of ammonia-extractable Ag correlated significantly with concentrations of HCl-labile Ag, across the variety of estuarine sediments (Fig. 5). About half of the HCl-extractable Ag appeared to complex to extractable organics. Interestingly, extraction of Ag from four sediments in the freshwater regions of these estuaries showed more HCl-Ag than would be expected from the ammonia-Ag. Thus, complexation with extractable organic materials may be less common in freshwater sediments than in estuaries. Reactions with sulphides cannot be eliminated as a possibility in these studies, although all sediments were collected from surficial layers of intertidal sediments, where sulphide concentrations are typically low. Silver sulphide has a strong formation constant, so where sulphides are abundant, AgS is likely to be important.

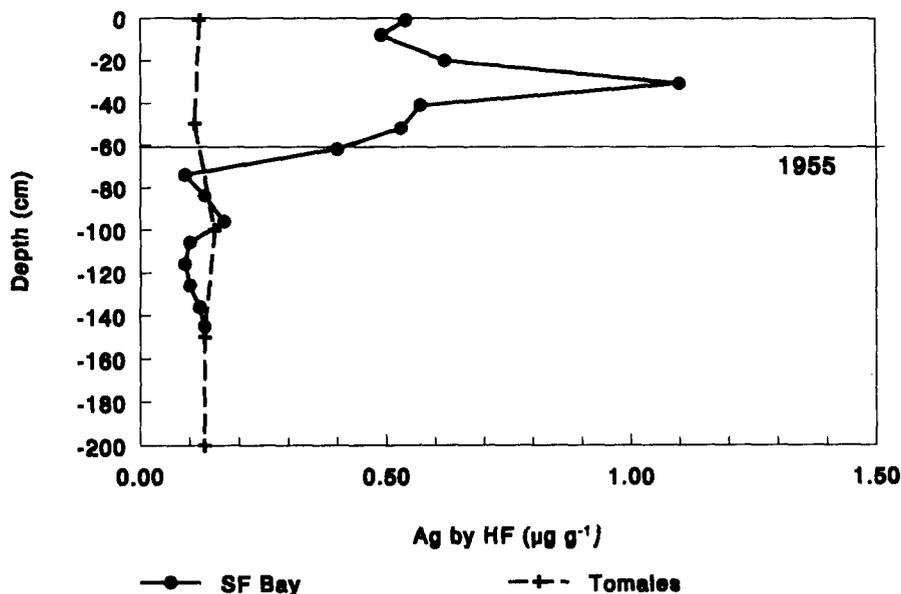


Fig. 3 Silver concentrations ($\mu\text{g g}^{-1}$ dry wt) in two cores as a function of sediment depth: San Francisco Bay, a system affected by human disturbance; and Tomales Bay, where the watershed is predominantly agricultural. The horizontal line represents the depth of penetration of ^{137}Cs in the San Francisco Bay core.

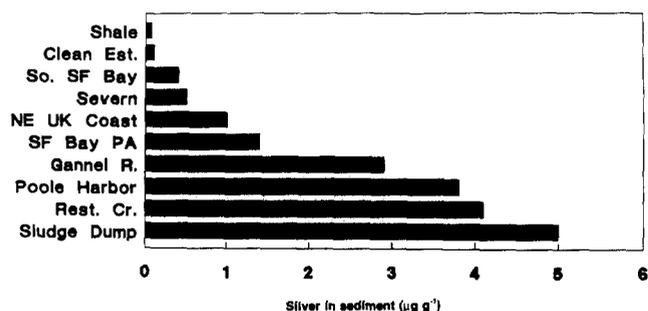


Fig. 4 Silver concentrations ($\mu\text{g g}^{-1}$ dry wt) in shale and in sediments from nine estuarine and marine ecosystems with different contamination (data from Bryan *et al.*, 1980, 1985; Luoma & Bryan, 1981; Luoma *et al.*, unpublished data). South SF Bay is South San Francisco Bay; NE UK Coast is the north-east coast of the UK; SF Bay PA is a contaminated site at Palo Alto in San Francisco Bay; Rest. Cr. is Restronguet Creek in the Fal Estuary in the UK.

Field studies and laboratory experiments show that Ag can accumulate in estuarine sediments despite the chloro complexation of dissolved forms. Aggregated coatings appear to play a role in enhancing the association with natural sediments in brackish and marine waters, although precise Ag associations within such materials are not known. Because Ag accumulates in sediments from contaminated environments, its storage, remobilization and bioavailability from sediments must be considered.

The bioavailability of silver

Several studies show that Ag is the most strongly bioaccumulated of the elements by at least some marine and estuarine invertebrates. Cherry *et al.* (1983) compared trace element enrichment factors for molluscs. The enrichment factor was defined as the ratio of element concentration in the molluscan digestive gland to the concentrations in seawater. Enrichment factors for Ag exceeded those of all other

elements. Bryan (1984) summarized studies where experimental uptake of dissolved forms was compared among metals. Ag, Hg and Cu were the most readily accumulated of the trace elements by phytoplankton, some types of seaweed, and oysters. Bryan (1985) also ranked trace metals by the ratio of metal concentrations in tissues to metal concentrations in sediments, using data from two estuaries in the UK. He compared tissues from three sediment dwelling species: the deposit feeding clams *Scrobicularia plana* and *M. balthica*, and the polychaete *Nereis diversicolor* (also a deposit feeder). Silver was the most strongly bioaccumulated of all the elements in all three species in these systems.

The strong bioaccumulation of Ag in estuarine and marine environments may at least partly result because the neutral chloro complex of Ag (AgCl^0) is highly bioavailable (Engel *et al.*, 1981). Sunda (1993) suggested that the low polarity of this complex facilitates its diffusion across biological membranes. Thus, two aspects of speciation are of great consequence to Ag bioavailability in the transition from freshwater to marine waters: free ion activity and neutral chloro complex formation. The neutral chloro complex is of predominant importance in marine waters because of its abundance and because free ion activity is so low. In addition, a contribution to bioavailability of other species of dissolved Ag has not been eliminated (Cowan *et al.*, 1993). For example, a neutral charge silver bisulphide complex (AgHS^0) apparently contributes a significant proportion of the activity of the element in brackish waters at free sulphide concentrations as low as $0.01 \mu\text{g l}^{-1}$ (0.2 nm kg^{-1} , see Cowan *et al.*, 1993). What is most notable about all the above reactions is that the speciation reaction that enhances Ag solubility and dispersion in estuarine/marine waters also enhances its bioavailability. In this way, Ag is an exception among the trace elements.

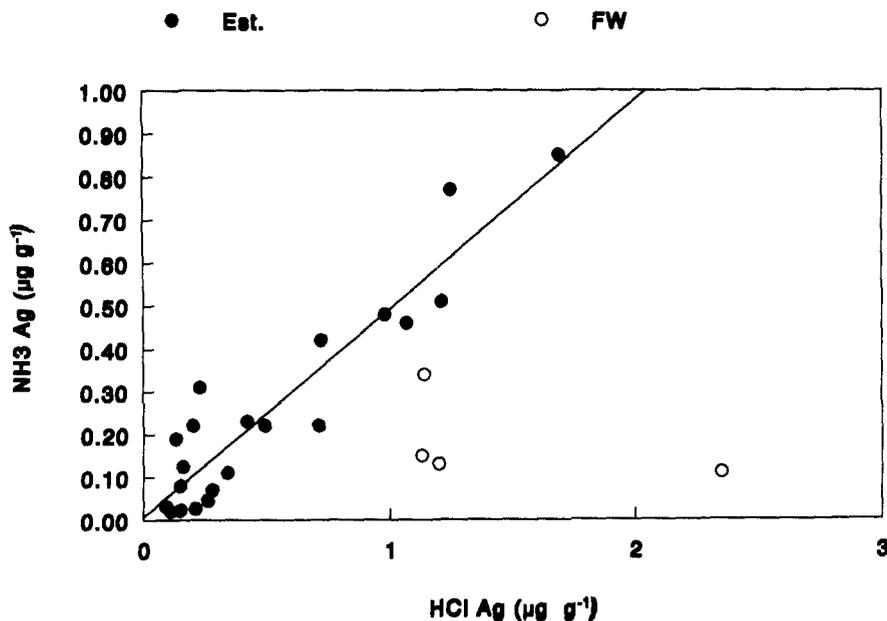


Fig. 5 Correlation of Ag concentrations ($\mu\text{g g}^{-1}$ dry wt) in 1 M ammonium extract (NH_3 Ag) with concentrations in 0.5 M HCl extractions (HCl Ag) among sediments from 17 estuaries from the UK. (Data from Luoma & Bryan, 1981; includes unpublished data collected in 1977.)

Silver uptake from contaminated particles. Results differ among studies that have assessed the bioavailability of Ag from particulate materials. Phytoplankton strongly bioconcentrate Ag (Fisher *et al.*, 1984). Thus, ingestion of food is a potentially important vector of Ag transfer to marine and estuarine herbivores. Some studies have shown that Ag can be assimilated or taken up from certain types of ingested food by oysters (*Crassostrea gigas*; Berthet *et al.*, 1992); mussels (*Mytilus galloprovincialis*; Ettajani *et al.*, 1992); and clams (*M. Balthica*; Luoma & Jenne, 1977; Harvey & Luoma, 1985). In contrast, Sanders *et al.* (1990) observed no detectable assimilation of Ag from different types of particulate materials by the oyster *Crassostrea virginica* or the shrimp *Palaemonetes pugio*. It is likely that Ag assimilation from particles differs among food types and among species, but quantitative generalizations have yet to be determined.

The bioavailability of Ag from contaminated sediments was tested with the deposit feeding clam, *M. balthica*. The clams used in the experiment had a history of moderate Ag exposure ($4 \mu\text{g g}^{-1}$ dry wt initial concentration of Ag in their tissues). Thus, only when HCl-Ag concentrations exceeded $1 \mu\text{g g}^{-1}$ dry wt in the sediments was net uptake observed (Fig. 6). At higher concentrations bioaccumulation was greater as Ag concentrations in sediments increased. Uptake was also determined when sediments were treated to remove iron oxides and organic materials. Clams held in chambers above the sediments showed no net uptake of the element for 23 days in any treatment; thus, dissolved Ag made a minimal contribution to bioaccumulation. After 10 days, significant Ag was bioaccumulated from the spiked, unextracted sediment (Fig. 7). No Ag uptake was detectable from the pre-extracted sediments. Net uptake was detected in both treatments at 23 days. Uptake was not comparable to

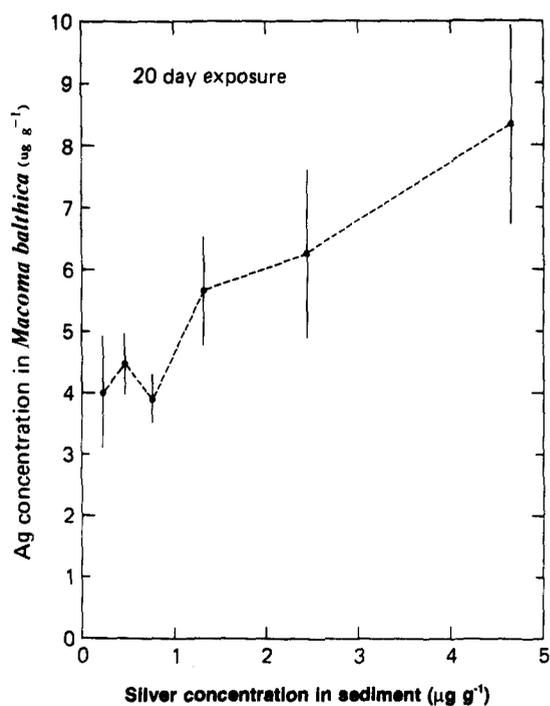


Fig. 6 Uptake of Ag by the clam *Macoma balthica* as a function of Ag concentrations spiked into oxidized estuarine sediments. All data are dry weights. Vertical bars are ± 1 SD.

that observed in natural settings (see later discussion), at least partly because of the limited duration of the experiment. The nature of the food available in the experiment may also have contributed to the limited bioaccumulation.

These experiments indicate that the factor that enhances Ag sorption to sediments, i.e. the complex labile coatings, also appears to enhance Ag bioavailability. The delayed uptake from the stripped sediments could have resulted from the development of

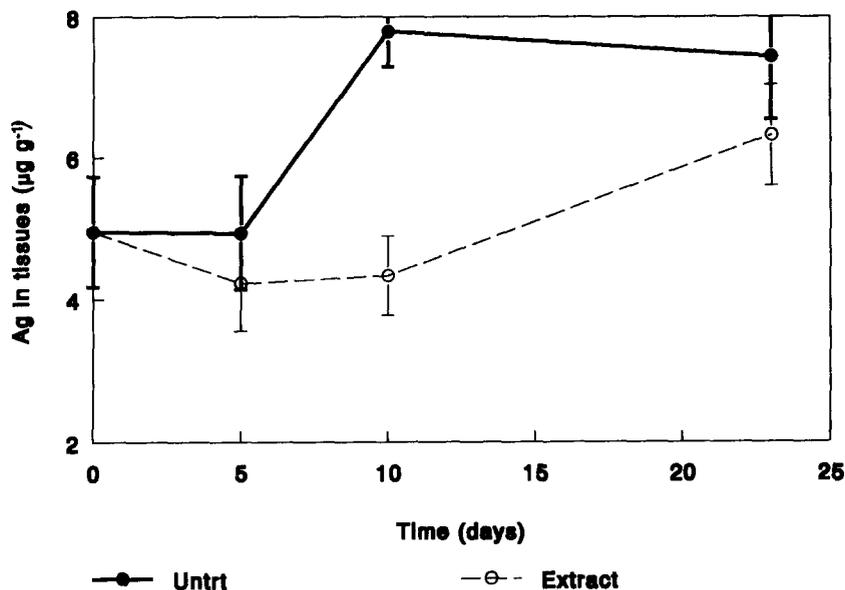


Fig. 7 Uptake of Ag by the clam *Macoma balthica* from oxidized estuarine sediments (closed symbols) and from sediments treated to strip iron oxide and bacterial coatings (open symbols). All data are dry weights. Vertical bars are ± 1 SD.

coatings on the sediments through time. Harvey & Luoma (1985) showed that no Ag uptake occurred from pure iron oxides spiked with ^{110m}Ag , but that uptake did occur if those sediments were coated with bacterial exudates. Harvey & Luoma (1984) also showed that such coatings can develop on clean sediment surfaces within 2 weeks.

Silver bioaccumulation in relation to concentrations in estuarine sediments. Luoma (1989) suggested that the bioavailable fraction of many metals in sediments may lie within the phase extractable by weak acid (0.5–1 M HCl), but that such an extraction was not necessarily a predictor of metal availability. Silver may be an exception to this generalization, in that HCl-extractable Ag in sediments may be a reasonable predictor of bioavailability, to at least some species.

Luoma & Bryan (1981) found a weak correlation between HCl-extractable Ag in sediments and Ag concentrations in the clam *S. plana*. Uptake appeared to be inhibited in *S. plana* from environments with very high Cu concentrations in sediments. When a multiple regression was calculated that included HCl-extractable Ag and Cu in sediments, 77% of the variance of Ag concentrations in *S. plana* was explained. Considering that the correlation occurred among nearly 50 stations in 17 estuaries, the strength of the correlation was remarkable. In support of the general inhibitory nature of Cu on Ag uptake, Bryan *et al.* (1983) showed that high concentrations of Cu in the diet of snail *Littorina littorata* inhibited Ag uptake. The interaction has only been shown experimentally for uptake from food. Studies of dissolved Ag and Cu uptake have shown either no interaction or a stimulation of Ag bioaccumulation by Cu exposure (Ettajani *et al.*, 1992).

A review of Bryan's surveys of English estuaries indicates that the deposit feeding clam *M. balthica* was not found in estuaries with high Cu concentrations in sediments. Thus, an inhibitory influence of very high sedimentary Cu on Ag bioavailability in this species

was not reported. Bryan (1985) showed a very strong correlation between Ag in *M. balthica* and HCl-extractable Ag in sediments (14 data points from British estuaries; $r^2=0.69$; $p<0.001$). Ag contamination is also widespread in San Francisco Bay; here, *M. balthica* is common, and copper concentrations in sediments are relatively low ($<100 \mu\text{g g}^{-1}$ dry wt). The Ag concentrations in *M. balthica* from San Francisco Bay followed the same relationship as those from the British estuaries, relative to HCl-Ag in sediments (Fig. 8). When the data from the two locations were

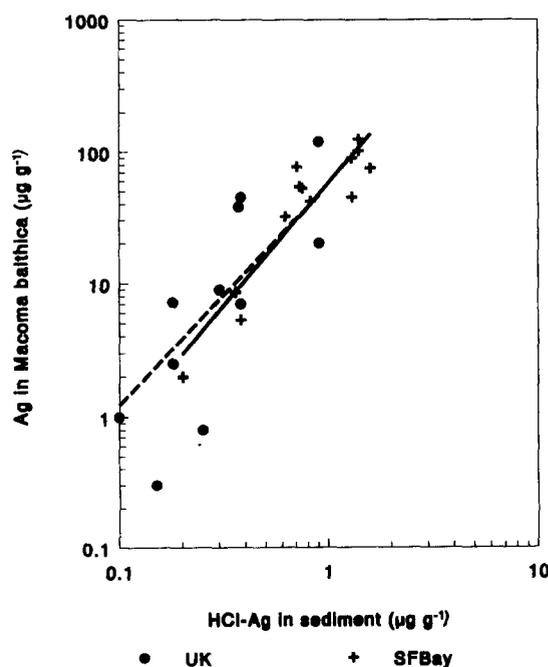


Fig. 8 Concentrations of Ag ($\mu\text{g g}^{-1}$ dry wt) in the tissues of *Macoma balthica* compared to concentrations in sediments extracted by 0.5 M HCl. Closed circles = UK estuaries from Bryan (1985); + = San Francisco Bay from Luoma & Phillips (1988) and Luoma *et al.* (unpublished data). The solid line is the best fit to San Francisco Bay data; the dashed line is the best fit to UK data. r^2 for entire data set is 0.80 ($p<0.001$).

combined, 80% of the variance in Ag concentrations in *M. balthica* was explained. The global nature of this relationship suggests it could be useful in predicting bioavailable sedimentary Ag. Neither HCl extractability nor the bioavailability of Ag have been studied in environments where sulphide deposition results in high concentrations of the element in sediments, but AgS is not highly soluble in HCl.

In the combined data from the British estuaries and San Francisco Bay, the slope of the relationship of Ag bioaccumulation to sedimentary Ag was 56. Small changes in Ag concentrations in sediments had great consequences with regard to bioaccumulation. Less than $2.0 \mu\text{g g}^{-1}$ dry wt of Ag in oxidized sediments resulted in $100\text{--}200 \mu\text{g g}^{-1}$ dry wt in the tissues of biota; concentrations which are rarely found in living animals. In San Francisco Bay, sediment concentrations of $\sim 1 \mu\text{g g}^{-1}$ dry wt have been associated with the bioaccumulation of Ag concentrations that lead to adverse sublethal effects (see later discussion).

Sediment-water exchange of Ag, as well as its availability from ingested particles, could contribute both to the correlations of Ag bioavailability with sediment concentrations, and the very strong bioaccumulation of the element. Bryan (1985) suggested that both dissolved and sedimentary ingestion were active pathways in Ag bioaccumulation by sediment-dwelling species. The experiments discussed above suggested that direct bioaccumulation of Ag from ingested particles can occur. Two lines of indirect evidence indicate a contribution of uptake from solution. Distributions of Ag amongst tissues of the oyster *Crassostrea gigas* (Berthet *et al.*, 1992) and the clam *S. plana* (Bryan & Uysal, 1978) followed a pattern that would be expected from dissolved uptake. Correlations were also observed between Ag concentrations in benthic animals and concentrations in the seaweed *Fucus vesiculosus* (Bryan, 1985). The most likely source of the element in seaweed was dissolved Ag. More advanced study is necessary to differentiate the quantitative contributions of the various uptake pathways under different conditions.

Perhaps because of the complex roles of different pathways in uptake, the bioavailability of Ag appears to be enhanced in the vicinity of sewage outfalls. Bryan and his colleagues were among the first to note that high Ag

concentrations in bioindicator species were observed in general in English estuaries having major sewage inputs (Thames, Severn, Clyde; Bryan & Langston, 1992). Bryan & Hummerstone (1977) found that biota from the West Looe estuary were less contaminated by Ag than were animals from the adjacent East Looe, although the levels of Ag in sediments were similar in the two. The source of contamination was mine waste in the West Looe, whereas sewage was the predominant source in the East Looe. Thomson *et al.* (1984) reported that Ag was highly concentrated in clam tissues near a sewage outfall in San Francisco Bay. The ratio of Ag in clam tissues compared to that in sediment was greatest nearest the outfall.

Several processes may contribute to these observations. If the association of Ag with particles near outfalls is slow, dissolved concentrations of Ag could be locally increased nearest the outfall. If availability is initially high from recently sorbed Ag, enhanced bioavailability from particles might be expected nearest the outfall. Sewage-derived particles may also contain the types of coatings that accentuate bioavailability, such as bacterial exopolymers accumulated in the treatment process (Harvey & Luoma, 1984). In contrast to the results near sewage discharges, Bryan (1985) observed a negative correlation between the level of Ag in the polychaete *N. diversicolor* and the Ag/organic matter ratio in the sediments of the Gannel estuary. This led Bryan & Langston (1992) to suggest that organic materials such as humic substances may suppress Ag bioavailability, while other organic materials such as exopolymers could enhance it. More specific study of such possibilities is needed.

Silver toxicity

In comparable bioassay tests, Ag is one of the three most toxic of the elements (with Cu and Hg) to invertebrates and algae in marine and estuarine environments (Bryan, 1984). At least some marine or estuarine life forms are sensitive to very low concentrations of Ag under bioassay conditions (Table 1). Bryan & Langston (1992) stated that 'the early life stages of bivalve molluscs are by far the most sensitive to Ag'. In addition, toxic and sublethal responses have been observed in early life stages of fish (the larvae of flounder) and in phytoplankton (dinoflagellates; see

TABLE 1
Selected examples of species and life stages that are highly susceptible to Ag toxicity.

| Species | Exposure/response | Toxicity ($\mu\text{g l}^{-1}$) | Reference |
|---|-------------------------------------|-----------------------------------|--------------------------------|
| <i>Crassostrea gigas</i> (embryo, larvae) | | 14 | Dinnel <i>et al.</i> (1982) |
| <i>Spisula solidissima</i> (surf clam) embryo | 1 h exposure, 48 h EC ₅₀ | 14 | Zoto & Robinson (1985) |
| <i>Acartia clausii</i> (adult copepod) | | 13 | Lussier & Cardin (1985) |
| <i>Crepidula fornicata</i> , snail (larvae) | 24 months, larval release | 10 | Nelson <i>et al.</i> (1983) |
| <i>Crassostrea virginica</i> (embryo) | 48 h LC ₅₀ | 6 | Calabrese <i>et al.</i> (1973) |
| <i>Paralichthys dentatus</i> , summer flounder (larvae, embryo) | | 5-8 | Cardin (unpublished data) |
| <i>Homarus americanus</i> , lobster (adult) | Enzymatic signs of stress | 6 | Calabrese <i>et al.</i> (1977) |
| <i>S. solidissima</i> (surf clam) (gamete) | 45 min | 6 | Eyster & Morse (1984) |
| Phytoplankton (natural population) | Enclosure-mesocosm | 5 | Sanders <i>et al.</i> (1990) |
| <i>Mytilus edulis</i> (embryo) | 72 h | <4 | Dinnel <i>et al.</i> (1982) |
| <i>Gymnodinium</i> spp., dinoflagellate | 2 days | 2-10 | Wilson & Freeburg (1980) |
| <i>Arbacia lixula</i> , sea urchin (embryo) | 52 h | 0.5 | Soyer (1963) |
| <i>Ilyanassa obsoleta</i> (embryonic devel.) | | <1 | Conrad (1988) |

Table 1). It is a reflection of the unique influence of Ag speciation that the Ag concentrations that cause toxicity are orders of magnitude lower than toxic concentrations of Cd in comparable tests (see comparisons in Bryan, 1984). Cadmium also forms chloro complexes in seawater, but those complexes are not biologically available.

The range of silver toxicities in Table 1 is < 1 to $14 \mu\text{g l}^{-1}$. These are low concentrations for the toxicity of any trace element. Nevertheless, widespread concentrations of Ag in the $\mu\text{g l}^{-1}$ range are rarely reported even in polluted natural water. The highest concentrations of dissolved Ag reported (using reliable chemical techniques) in the open waters of San Francisco Bay are $0.025 \mu\text{g l}^{-1}$ (Smith & Flegal, 1993). Thus, a disparity exists between experimental toxicity and natural dissolved concentrations of Ag. This is balanced by the likelihood that toxicity tests, because of their simplistic designs, are less sensitive than natural systems. For example, the tests do not employ the most sensitive species from nature (and sensitive life processes are not fully known). Experimental toxicity tests rarely account for multiple pathways of exposure, nor can most designs consider the complex, often secondary interactions that influence toxicity in ecosystems (Luoma, in press). The toxicological uncertainties imposed by chemical disparities and biological insensitivities suggest that no conclusion can yet be drawn about Ag toxicity in polluted natural waters.

The difficulties of specifically proving that a pollutant is the sole cause of ecological damage with field studies are evidenced by the contradictory results that are often observed. Martin *et al.* (1984) found a reduced condition index and reproductive impairment in mussels transplanted into south San Francisco Bay. The stress was correlated with Ag concentrations, but also with several other potential sources of damage. Worrall & Widdows (1983) could not demonstrate ecological damage in an Ag-contaminated mudflat. Sublethal symptoms of toxicity were observed in bivalves on an Ag-contaminated mudflat in San Francisco Bay, but community change was difficult to identify (Luoma & Phillips, 1988).

Only a few sensitive pollutant-specific approaches are available for proving the effects of metals in natural systems (Bryan *et al.*, 1987; Luoma & Carter, 1991). Contaminant-specific responses to Ag are not well-documented, and may not occur. Some general sublethal signs of stress might be expected. Adult bivalves appear to sequester (and presumably detoxify) Ag in metal-binding proteins (George *et al.*, 1986; Johansson *et al.*, 1986), and in sulphide-rich granules in amoebocytes and the basement membranes of cells (Martoja *et al.*, 1988). Occurrence of Ag in these forms may be a precursor to significant physiological damage. Premature release of reproductive products (Martoja *et al.*, 1988), reduced numbers of larvae (Nelson *et al.*, 1983), decreased storage of glycogen necessary for reproduction (Martoja *et al.*, 1988; Luoma *et al.*, unpublished data) and reduced growth (Calabrese *et al.*, 1984) have all been suggested in toxicity tests or Ag-contaminated populations. Tolerance to Ag (Cain &

Luoma, unpublished data), as well as biochemical shifts in the cytosolic distribution of Ag, are more subtle indicators of possible stress that have been reported (Johansson *et al.*, 1986). Better knowledge of which species are most vulnerable to Ag and their biochemistry, physiology and life history responses to the metal are required before sensitive determinations of Ag toxicity in nature will be possible.

Conclusions

The chemistry, bioavailability and toxicity of Ag all contribute to concern with respect to this element as a pollutant in marine and estuarine waters. Natural concentrations of Ag in seawater are very low ($1-3 \text{ pM}$; $0.1-0.3 \text{ ng l}^{-1}$), as they are in oxidized sediments ($0.1 \mu\text{g g}^{-1}$ or 100 nM kg^{-1} dry wt). As a result, relatively small anthropogenic inputs result in substantial environmental enrichment. Anthropogenic contamination can result in Ag concentrations in estuarine and marine waters that are 100–300 times higher than natural concentrations (Sanudo-Willhelmy & Flegal, 1992). Formation of a stable chloro complex favours Ag dispersal. Sequestration by sediments also occurs, with relatively slow kinetics. Silver enrichment in anthropogenically-contaminated estuarine sediments can reach concentrations 50 times higher than natural, although this is an absolute concentration of only $5 \mu\text{g g}^{-1}$ dry wt.

Silver is unusual, in that both the dominant speciation reaction in seawater and processes important in sorbing Ag in sediments favour enhanced bioavailability. The abundant chloro complex is available to biota and the amorphous aggregated coatings enhance Ag accumulation in sediments as well as Ag uptake from sediments by deposit feeders. In nature, bioavailability correlates with an easily extractable fraction of Ag that accumulates on contaminated particles.

In estuaries, the bioaccumulation of Ag increases as a power function of element concentrations in sediments. One unit of Ag contamination in oxidized sediment results in 56 units of Ag bioaccumulation in the estuarine clam *M. balthica*, over a wide range of estuaries. Thus a little Ag contamination in a marine and estuarine environment has great consequences for Ag bioaccumulation in at least some organisms.

The toxicity of Ag is the primary source of concern in marine environments. Toxicity for sensitive marine species occurs at absolute concentrations as low as those observed for any non-alkylated metal. Low Ag concentrations are toxic partly because bioaccumulation increases so steeply with contamination. Thus, the environmental window of tolerance to Ag in estuaries could be narrower than for many elements. It may also be narrower in estuaries than in freshwater. Too few studies have considered Ag toxicity in natural systems. Nevertheless, it is clear that the effects of Ag in nature deserve considerably more careful study than has been assigned to them to date.

Sediment core data shown here were collected in a multi-investigator project co-led by S. L. and Alexander van Geen. Cores were obtained by Roberto Anima of USGS and Tim Hollibaugh (Tomales Bay) of Romberg Marine Center, Tiburon, CA. Silver analyses were conducted by Robin Schaeneman of USGS. Dating information was conducted by Dr M. Baskaran, Texas A&M. Detailed information on core chronologies are in preparation. The second set of experiments on Ag sorption (using sediments extracted by hydroxylamine hydrochloride and acetic acid) was conducted by Virginia Elrod, now with Moss Landing Marine Laboratories, Moss Landing, CA.

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