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THE INFLUENCE OF SALINITY ON COPPER ACCUMULATION BY THE AMERICAN OYSTER, CRASSOSTREA VIRGINICA, AND THE SOFT SHELLED CLAM, MYA ARENARIA

UM-SG-TS-83-08

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With the enormous release of metals discharged into the Chesapeake Bay each year from heavy industry and sewage treatment plants as well as run-off from farms and urban areas comes potential hazards to estuarine life. Just what those hazards are--the potential toxicity that results from the availability of metals--depends in part on the chemical changes that metals undergo; and those changes are affected by environmental factors, which are subject to seasonal variation, for example, pH, temperature and salinity. The potential hazards also depend on the particular organisms: animals like adult oysters and clams themselves differ in the amounts of metals they will accumulate under varying environmental conditions.

In an attempt to differentiate among environmental influences on the bioavailability of metals, the authors conducted a series of experiments in which they analyzed the effects of different salinity regimes on copper availability in two commercially important organisms in Chesapeake Bay, the American oyster, Crassostrea virginica, and the soft-shelled clam, Mya arenaria. They have observed that at low salinities, both oysters and clams accumulate more copper than they do at high salinities; and between the two, clams accumulate more copper than do oysters. The authors present a number of competing explanations that take into account the effect of salinity on the organisms themselves. The implications of this research could be of use to studies that evaluate the impact of siting industrial complexes within low salinity regions of the estuary; it should also be of value to monitoring studies that make use of molluscs as indicators of marine pollution.

--The Editors

INTRODUCTION

Previous studies suggest that the bioavailability of certain trace metals to estuarine organisms is salinity dependent. Higher tissue levels of trace metals have

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been found in organisms harvested from progressively fresher regions of many estuarine systems. This concentration gradient has been reported for many organisms including bivalve molluscs (Huggett et al. 1973; Denton and Burdon-Jones 1981), crustaceans (Vernberg et al. 1977) and polychaetes (Bryan and Hummerstone 1971). Laboratory studies indicate that salinity affects the rate of trace metal accumulation and toxicity (Phillips 1976; Jackin et al. 1977; Wright 1977). Variations in salinity may, in part, explain the spatial and temporal variation of trace metal levels observed in many estuarine species.

Several mechanisms have been proposed to explain this salinity effect on the uptake of trace metals by estuarine biota. For example, it has been postulated that salinity affects the biological activity or physiological processes directly, leading to alterations in metabolic rates, filtration rates and feeding (Bass 1977). Alternatively it has been postulated that the "salinity effect" may result from the influence of salinity on the chemical speciation of trace metals. The bioavailability of "dissolved" copper to Crassostrea virginica is reduced by organic complexation (Zamuda and Sunda 1982); and changes in salinity can alter the level of organic complexation of trace metals and in turn lead to changes in bioavailability. Others have suggested that the "salinity effect" is due to changes in the ratio of major ions to trace metals and its subsequent impact upon co-transport and competition at uptake (Phillips 1976; Bryan 1976; Wright 1977). If relatively non-specific ion transport mechanisms are responsive to ambient calcium availability, then salinity may affect trace metal uptake. Unfortunately, little progress has been made in detemining the relative importance of these postulated mechanisms.

Our research here has examined the influence of salinity on copper accumulation by the American oyster, Crassostrea virginica, and the soft shell clam, Mya arenaria. A chemically defined seawater medium was used to investigate the effects of salinity on the chemical speciation of copper and its biological availability. Exposure solutions were selected to achieve similar values of cupric ion activity within a range of salinities, using different combinations of NTA and total dissolved copper.

MATERIALS AND METHODS

The oysters for these experiments were hatchery reared and were genetically similar, thereby reducing the variability among organisms collected from the field. Soft shell clams were reared in the laboratory and, with the oysters, were maintained in a flow-through seawater system prior to use in the salinity-copper accumulation studies. Two sets of experiments were conducted. Experiment I examined copper accumulation in oysters maintained in synthetic seawater. Experiment II compared the effect of salinity changes on copper accumulated by oysters and soft shell clams maintained in filtered natural estuarine water.

Experiment I: Oysters were exposed to a range of dissolved copper and cupric ion activities at different salinities in synthetic seawater. The concentration of the major seawater salts in the synthetic medium are those of S.O.W. (PWPCA 1969). Trace metal impurities associated with the reagent grade salts were reduced to acceptable levels by Chelex-100 batch ion exchange treatment. Salinities were adjusted with the addition of distilled-deionized water to achieve treatment salinities of 10 and 30 ppt.

Oysters were exposed for 7 days to a range of total dissolved copper concentrations (10 and 30 μg Cu L^{-1}), and to a range of cupric ion activities (10^{-10} and 10^{-9} Cu^{2+}) in metal-buffered medium.

Control treatments consisted of synthetic seawater without the addition of trace metals or chelators. Ten oysters were sampled on Day 0 (18 Oct 82) to obtain initial tissue concentrations and 5 individuals removed from each treatment tank at the end of 7 days for metal analysis. The 4 liter exposure medium in each polyethylene tank was changed daily. The tanks were aerated and the pH and salinity of each exposure tank measured periodically. Salinity and pH did not vary with time of exposure. The pH for the salinity treatments of 10, 20 and 30 ppt were 7.9, 8.0 and 8.2, respectively.

Oyster tissues were analyzed for trace metal concentrations by removing the soft tissue and determining the dry weight by oven drying (105°C) for 24 h. The tissues were digested in hot concentrated nitric acid, evaporated to dryness, redissolved in 0.25 N HCl and analyzed for copper by flame atomic absorption spectrophotometry.

Cupric ion activities in exposure media containing NTA were determined from thermodynamic calculations described by Zamuda and Sunda (1982). The value $10^{-1.7}$ ($10^{-1.5}$ used for pH 7.8 and 10 ppt salinity) is the total cupric ion activity coefficient at pH 8.1 as based on the interaction of copper with inorganic ions in 30 ppt seawater (Sunda and Gillespie 1979; Zirino and Mamaimoto 1972; Mantoura et al. 1978).

Experiment II: Oysters and soft shell clams were exposed to a range of dissolved copper (10 and 30 μg^{-1}) and cupric ion activities of (10^{-10} or 10^{-9}M Cu^{2+}) in filtered estuarine water. (Natural estuarine water was sequentially filtered through a 5 µm and 100,000 nominal molecular weight membrane filter.) Salinities were adjusted with distilled-deionized water to yield final salinities of 10 and 20 ppt.

Shellfish were exposed for 7 days to the various treatments in a static system as described in Experiment I. Ten oysters, as well as ten clams were sampled on Day 0 (6 Dec 82) and on days 3 and 7, five individuals were removed from each treatment tank and analyzed for metal accumulation as previously described.

RESULTS

Copper concentrations in the tissues of Crassostrea virginica and Mya arenaria increased under conditions of reduced salinity. Results of Experiment I indicate that copper accumulation by oysters in synthetic seawater medium increased with: (1) increases in dissolved copper concentration, (2) increases in cupric ion activity, and (3) decreases in salinity. Similar results were obtained in Experiment II which examined copper accumulation in oysters and soft shell clams using natural filtered estuarine water in place of synthetic seawater media. Copper accumulation was significantly greater for oysters at 10 ppt salinity in Experiment I than Experiment II. This variation in accumulation rate may be due to differences in size of the oysters used in the two studies. Oysters used in Experiment I were significantly smaller (0.563 $\pm$ .030 g dry weight, $\bar{x} \pm 1$ S.E.).

Copper accumulation rates for soft shell clams were generally significantly greater than that for oysters, the result perhaps of differences in valve closure between these two molluscs; in contrast to oysters, the soft shell clams are incapable of full valve closure and can only close the mantle edges and siphon tubes by muscle action (Shumway 1977).

The relative increase in copper accumulation rate for oyster and soft shell clams associated with decreasing salinities was generally greater at the lower dissolved copper and cupric ion activity treatments. For example, at a total dissolved copper concentration of 10 µg Cu L^{-1}, oysters accumulated 62.5% less copper when the salinity

was increased from 10 ppt to 20 ppt. In contrast, at a dissolved copper concentration of 30 µg Cu L^{-1}, the reduction in relative copper accumulation rate associated with the same change in salinity was 29.7%.

DISCUSSION

The accumulation of copper by the oyster C. virginica and the soft shell clam, Mya arenaria was significantly influenced by salinity. Accumulation rates increased with reductions in salinity at similar dissolved copper concentrations. These results are in agreement with previous studies of trace metal-salinity interactions. Denton and Burdon-Jones (1981) reported that accumulation rates of Cd, Hg and Pb by oysters were significantly greater in low salinity water. MacInnes and Calabrese (1979) suggested that low salinity acted synergistically with copper, resulting in increased abnormalities among Crassostrea virginica embryos. This interactive effect between salinity and metal concentrations, resulting in increased embryo toxicity, has also been demonstrated for copper and silver utilizing the Pacific oyster, Crassostrea gigas (Coglianese 1982). Laboratory studies indicate that reductions in salinity result in increased metal accumulation or toxicity for many marine species, including Hg in bay scallops, Argopecten irradians (Nelson et al. 1977) and in clams Rangia cuneata (Olson and Barrel 1973). Cu in estuarine crabs, Cancer irroratus and Carcinus maenas (Thurburg et al. 1973); and Cd in mussels, Mytilus edulis (George et al. 1978).

In addition to laboratory evidence, several field studies have also demonstrated higher concentrations of metal in organisms sampled from the lower salinity regions of their distribution. For example, Huggett et al. (1975) reported a metal concentration gradient in oysters sampled from Virginia and North Carolina estuarine systems, with progressively higher concentrations of either copper or zinc in progressively fresher waters. While the authors suggested that this pattern was the consequence of oysters in fresher water being closer to the metal source, their metal-water chemistry data, and data from other researchers, indicate that the concentration gradients found in oysters were not reflected in a similar gradient in the water (Cross et al. 1970; Cronin et al. 1974). Gradients of lead tissue concentrations in Mytilus edulis (Shulz-Baldes 1972), and Cu, Zn and Cd levels in oysters (Mackay et al. 1975) have also been attributed to dilution of polluted fresher water with seawater although water chemistry data were unavailable.

Gradients in concentrations of dissolved and particulate metals may occur in many estuarine systems and be partially responsible for the observed inverse relationship between salinity and tissue metal concentrations. Yet, this would not explain our laboratory results in which Cu accumulation by oysters and soft shell clams was greater at lower salinities under conditions of constant dissolved copper concentrations. Thus, other factors must be interacting to control the availability of trace metals to estuarine biota.

Various explanations have been proposed to describe the relationship between trace metals accumulation and salinity. Change in salinity may result in several physiological effects on oysters and soft shell clams, which may influence the accumulation of trace metals. Bass (1977) demonstrated that the oxygen consumption in oyster tissue increased in diluted seawater. Denton et al. (1981) suggested that under such conditions increased ventilation rates or filtration rates may increase rates of metal uptake.

George et al. (1976) suggested that an increase in cadmium uptake with decreasing salinity was due to the effect of osmolarity of the surrounding medium. These osmotic effects might be due to increased ion fluxes or a physical effect of swelling of the cell membrane causing unmasking of a carrier or transmembrane pore under conditions of reduced salinities. However, a true euryhaline species will show neglegible changes in cell volume under osmotic stress (Florkin and Schoffeniels 1969). Thus, increased metal uptake due to swollen cells resulting from reduced salinity appears uncertain.

Increased ion fluxes associated with calcium transport may, under conditions of reduced salinity, play a significant role in altering trace metal accumulations. Studies examining Crassostrea virginica (Lynch and Wood 1966) and Mya arenaria (Virkar and Webb 1970) suggest that regulation of intracellular free amino acids (FAA) prevents an excessive swelling of cells in response to changes in salinity. The total FAA pool size decreases in response to decreased salinity and increases in response to elevated external osmotic concentrations (Otto and Pierce 1981). It appears that intracellular FAA regulation and hence intracellular volume regulation response requires a critical external concentration of calcium. In addition, calcium binding to the surface cell membranes has been shown to affect permeability characteristics (Walts and Pierce 1978). It is possible that the interaction between calcium and intra- and extra-cellular osmoregulatory mechanisms in bivalves in response to salinity changes may result in changes in trace metal uptake.

Rather than physical alteration of the cellular membrane due to salinity changes, increased rates of accumulation of metals at lower salinities may be due to competition between cations for binding sites. At increased salinities, the more abundant cations in seawater (i.e., calcium, magnesium) may out-compete trace metals for binding sites. The effects of hardness and cation competition on toxicity of trace metal ions are well documented. For example, Romeril (1971) reported that oyster uptake of ^{65}Zn decreased with the addition of iron and cobalt. The possibility of ion co-transport and competition for uptake has been suggested, in particular with reference to uptake of trace metals, in response to ambient calcium availability. Wolfe (1970), for example, postulated that zinc may be assimilated from the environment along with calcium by a relatively nonspecific ion-transport mechanism. With reduced calcium concentration in lower salinity waters, organisms may have to extract calcium more "efficiently" at lower salinities and may also concentrate greater quantities of other cations such as copper and zinc.

In addition to the potential effects of salinity upon the physiological processes of estuarine organisms, salinity may also influence the chemical speciation of trace metals which, in turn, would affect their bioavailability. The accumulation of dissolved copper by marine organisms appears to be related to the free cupric ion. Such free ion dependence has been demonstrated for the oyster (Zamuda and Sunda, 1982) employing the use of cupric ion buffer systems in which different concentrations of synthetic chelators allowed the maintenance of cupric ion activities at levels which were representative of environmental levels. Thus, biological response to dissolved trace metals is a function of the free metal ion which is determined not only by the total dissolved metal concentration but also by the extent of metal complexation to both organic and inorganic ligands. Because of the strong affinity which copper has for organic ligands, its chemical speciation in most estuarine systems is expected to be dominated by organic complexes (Mantoura et al. 1978; Gillespie and Vaccaro 1978). The extent of this organic compexation will be a function of the concentration and composition of dissolved organic compounds, pH and salinity. However, decreased salinity will tend to increase organic complexation of copper and thus decrease cupric ion activity, due to decreased concentrations of calcium and magnesium which compete

with trace metals for available chelation sites (Mantoura et al. 1978). In addition, the rivers usually have higher concentrations of dissolved organic matter than seawater which would further favor the organic complexation of copper with decreased salinity. Thus, if the effect of salinity on copper speciation is through its influence on organic complexation we might expect decreased rather than increased copper accumulation, with decreased salinity.

That the salinity effect is not the sole result of changes in the cupric ion activity is evidenced by the results of our studies examining copper accumulation by bivalves at similar cupric ion activities for a range of salinities. Our results indicate that for the range of salinities of 10-30 ppt, under conditions of similar cupric ion activity, both oysters and soft shell clams, accumulated significantly more copper at progressively lower salinities. Changes in copper accumulation rates as affected by salinity were greater for the higher cupric ion activities. Thus, under conditions of elevated copper availability (increased anthropogenic input associated with industrial actvity), salinity will exhibit a greater impact upon the bioavailability of copper than under normal environmental conditions. In addition, although a very low level of dissolved metal alone may not affect estuarine organisms, alteration in salinity could intensify the accumulation and toxicity of low metal levels through increased bioavailability at low salinities. This may be especially relevant for the more sensitive life stages of estuarine organisms such as larvae and embryonic stages.

Monitoring programs that use bivalve molluscs such as oysters and clams as bioindicators of marine pollution need to consider the influence of environmental variables such as salinity upon trace metal bioavailability. Temporal and spatial variability may be as much a function of natural changes in salinity as it is a reflection of increased inputs of trace metals to estuarine systems. In addition, attempts to assess pollution stress from measurements of environmental metal levels must consider other environmental parmeters: factors that may influence the chemical speciation of trace metals and factors that may effect the physiological processes of the organisms (temperature, salinity, particulate concentrations).

Water quality criteria must be more ecosystem specific. Criteria established to protect organisms from metal stress in river water may be overly protective in marine systems. Similarly, criteria based on biological toxicity studies conducted in marine waters may underestimate the potential toxicity under estuarine conditions.

With regard to industrial and urban development within coastal areas, our studies indicate that the siting of discharge points of metals into coastal waters should consider the influence of salinity on metal availability. For example, the siting of an industrial complex within the lower salinity regions of estuarine systems rather than the higher salinity regions may produce a high degree of metal stress upon estuarine organisms as a result of increased metal loading and the "salinity effect." Our results would suggest that maximum protection (from trace metal effects) to oysters, soft shell clams and perhaps other estuarine organisms would be provided by restricting industrial siting and discharges to the higher rather than the lower salinity regions of estuarine systems.

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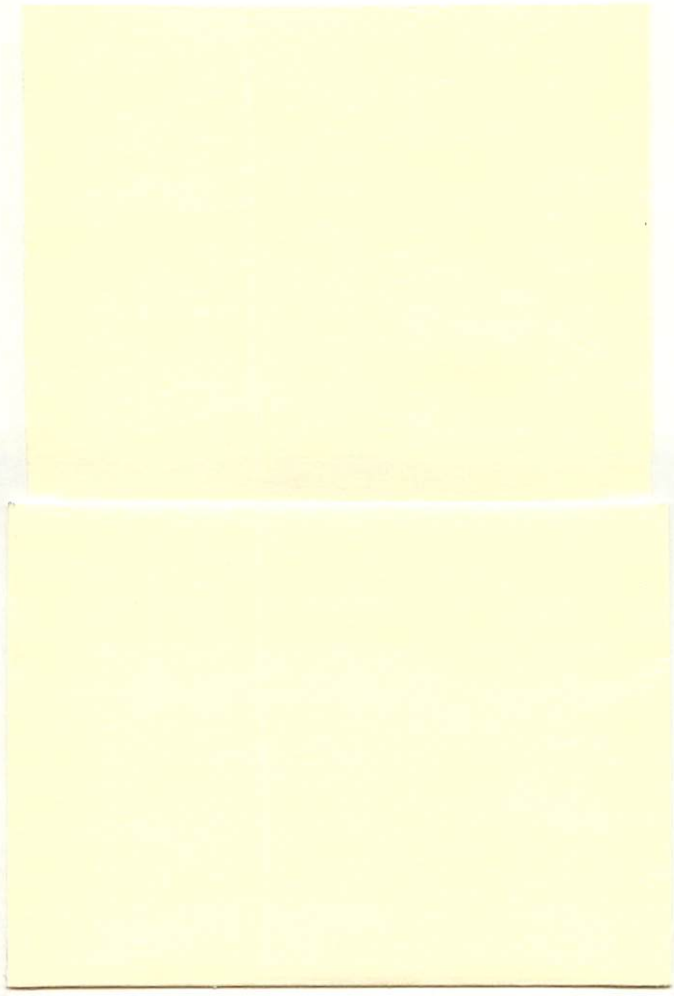
ACKNOWLEDGMENTS

This research was made possible by grant #NA81AA-D-00040, project #R/P-5, awarded by the National Oceanic and Atmospheric Administration to the University of Maryland Sea Grant College. For copies of the final report from which this research note is adapted, contact the Communications Office, Maryland Sea Grant College, 1224 H.J. Patterson Hall, College Park, MD 20742.



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